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Fine structure with regard to sperm functions of the ductus ejaculatoris duplex of the male reproductive tract of *Heliothis armigera* (Hübner) (Lepidoptera: Noctuidae)

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Abstract. The 'S' shaped duplex gland, the shortest among the male reproductive glands, ultrastructurally exhibits only a single cell type, characterized by the presence of multishaped rough endoplasmic reticulum, polymorphic Golgi bodies and vesicles. The frequent mode of secretion appears apocrine, with occasional merocrine and holocrine. Continuous with the height of secretory activity, the epithelia degenerates leaving only basement membrane and muscle layers.

Keywords. Apocrine; duplex; endocytosis; holocrine; merocrine; phagosome.

1. Introduction

Numerous studies have been made on the structure of the reproductive tracts in Lepidoptera (Norris 1932; Mehta 1933; Musgrave 1937; Williams 1940; Callahan and Cascio 1963; Tedders and Calcote 1967; Davis 1968; Fatzinger 1970; Thibout 1971; La Chance *et al* 1977; Brits 1978; Buntin and Pedigo 1983; Davey 1985; Scheepens 1986; Amaldoss 1987a, b). Ultrastructure studies of the Lepidopteran male reproductive tract are restricted to a few (Riemann and Thorson 1976a, b, 1979a, b; Lai-Fook 1982a, b, c, d, e; Amaldoss 1987a, b). There has been no ultrastructure study made on the male tract of *Heliothis armigera*. Therefore the present paper describes the fine structure of the duplex gland of *H. armigera*.

2. Materials and methods

Adult moths (1–2 day old) used in this experiment were reared in an artificial medium at 25°–28°C; 12:12 dark and light environment in a Long Light Company, Taiwan, growth chamber; RH 60±5%. Artificial diet Biomix 9787 (USA), was in usage. The male reproductive tract was dissected out in Weevers (1966), lepidopteran saline and duplex glands were safely separated and fixed in 2% glutaraldehyde, 0·1 M phosphate buffer (pH 7·4) for 3 h. After washing the tissue thoroughly in the same buffer, it was post fixed in 1% osmium tetraoxide (OSO_4) for 1·5 h. The tissue was washed again thoroughly in the same buffer and was dehydrated through a graded alcohol series and embedded in Spur EPON 812. Thin sections were cut in a Reichert, Austria, OM3 ultramicrotome. Thin sections were double stained in uranyl acetate for 5 min and lead citrate for 40 s. They were then viewed under JEOL JEM100S transmission electron microscope (TEM) and micrographs were taken.

Duplex glands of two days old virgin and mated males were dissected out in lepidopteran saline and were fixed in 2% glutaraldehyde, 0·1 M phosphate buffer at

4°C for 3 h. After thorough washing in the same buffer, the tissue was post fixed in 1% OSO_4 for 1·5 h, after which the tissue was washed again thoroughly in the same buffer and dehydrated through a series of alcohol. Following critical point drying, the tissue was coated with gold and examined with JEOL, JSMIS scanning electron microscope (SEM).

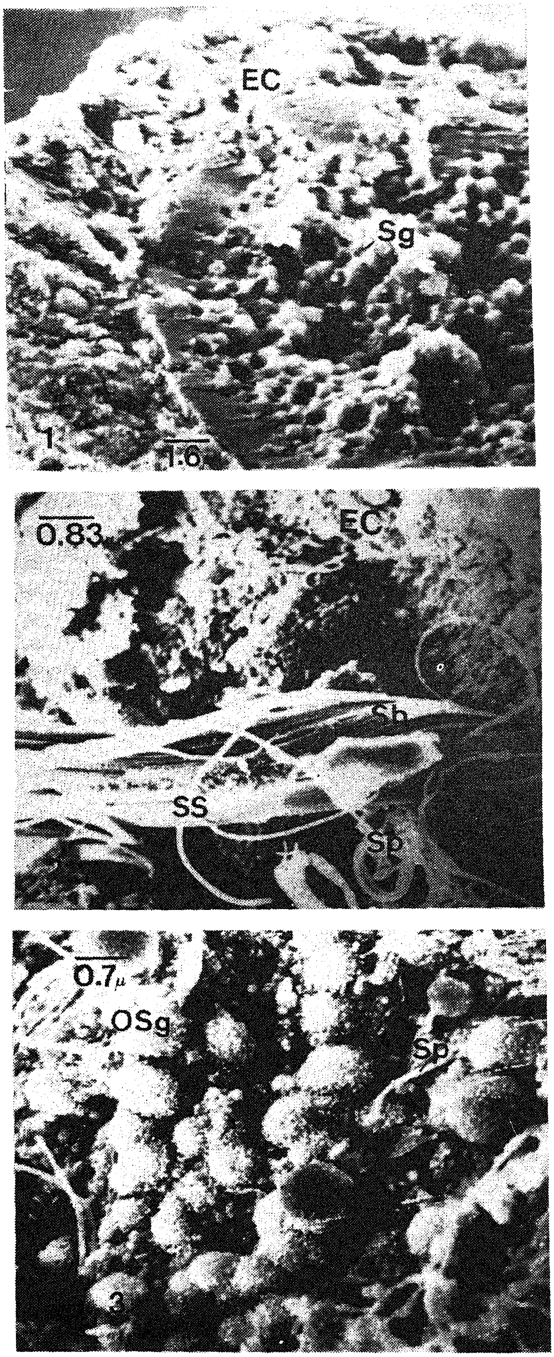
3. Results

Duplex are the shortest in the male reproductive tract of *H. armigera* and are paired, white and opaque glandular ducts. Duplex continue anteriorly by a pair of accessory reproductive glands (ARG) and continue posteriorly by an unpaired ductus ejaculatoris simplex (ejaculatory duct or simplex). Mid way through these glandular duplex arise a pair of thin extensible vasa deferentia (VD). At eclosion, duplex appear white and transparent. As it gains more secretory materials and receives sperm from the testes via VD, the glands appear full, white and opaque. The empty duplex measures about 7 mm in length and 1 mm in diameter in *H. armigera* but becomes distended when filled with a mixture of many types of secretion and spermatozoa. The region between the ARG and VD entrance at the duplex is shorter in comparison with a longer region between the VD entrance and simplex. Morphologically, duplex appear 'S' shaped and is easily collapsible resulting in the expulsion of spermatozoa and the seminal fluid. Histologically, the duplex appear to have a robust muscle wall and has a tall columnar epithelium. The nuclei are basally located and very large. Cytoplasm appears very granular and dense. The lumen exhibits discrete secretory globules.

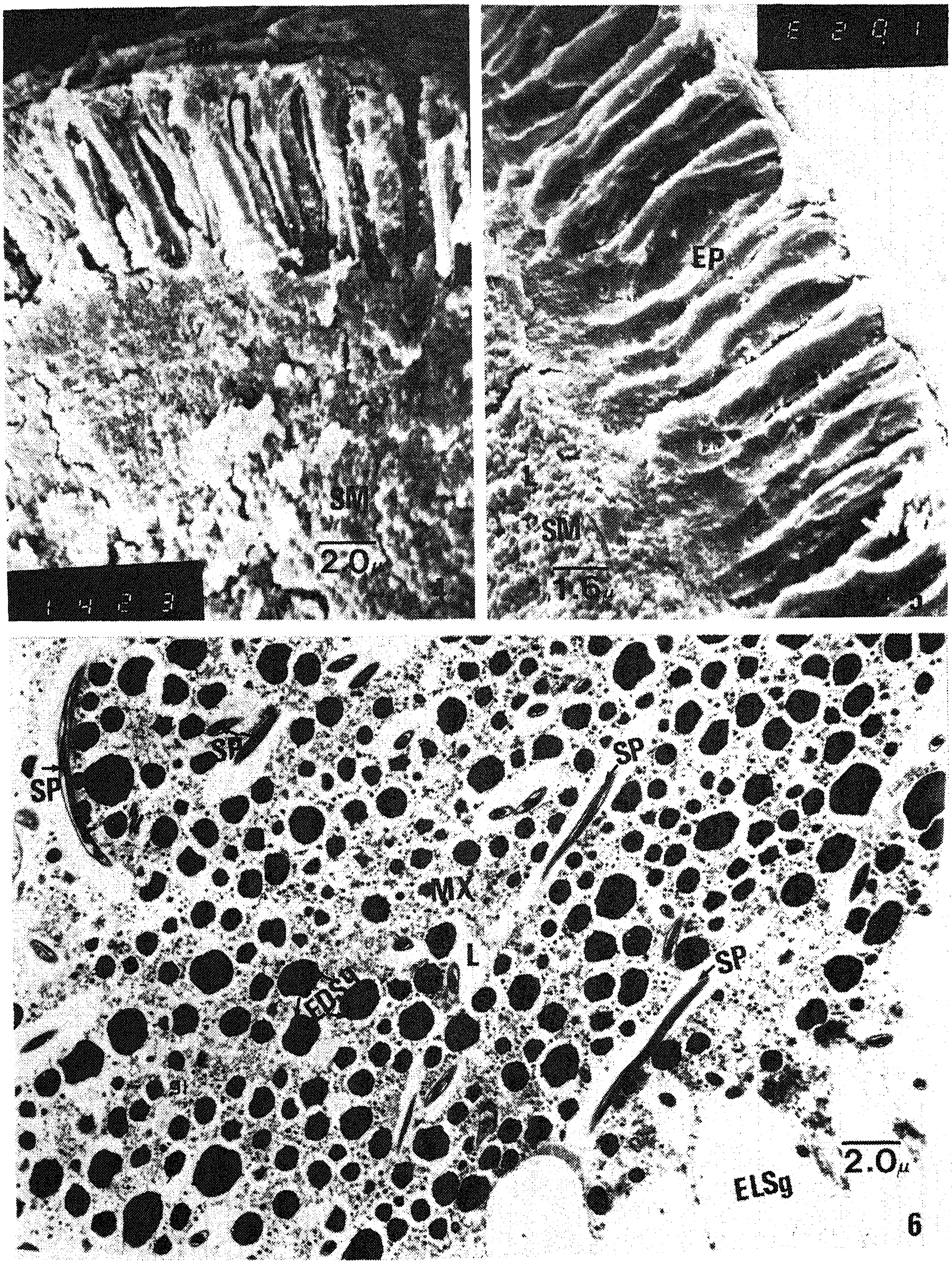
Ultrastructurally, the secretory epithelium (figure 7) is surrounded by a prominent basement membrane (BM), followed by a thick, robust circular inner muscle layer (CM) and moderately thick robust outer longitudinal muscle layer (LM). The muscle layers are profusely supplied with tracheae (Tr) and nerve endings (Ne). The Tr reach beyond the muscle layers into the basement membrane-epithelia. However, they do not penetrate deeply into the secretory epithelium. Axons containing neurosecretory particles can be seen in the muscle layers. All along the basement membrane into the basal epithelia, a number of invaginations (Biv) rise and lead into the epithelia. The secretory epithelium is made up of only a single cell type. However, it exhibits asynchronous secretory activity in the adjacent cells within epithelia of duplex with clear demarcation of their cell boundaries by cell membrane (CM) (figure 7). The cytoplasm is dense and granular. The presence of multishaped rough endoplasmic reticulum (rER) is the characteristic of the epithelium. rER may assume such forms as vesiculate (VER), flattened lamellae, distended sacculus and finger like configuration (FC) (figures 7, 8, 13–15). The presence of dense cored vesicles or particles is another common feature of the epithelia. The presence of numerous mitochondria (M) particularly at the sites of synthesis and of various other materials (figures 13–15, 18) are significant. Another unique feature of the epithelia is polymorphism of Golgi bodies (GB). Depending on the type of synthesis, site of their synthetic activity they are involved in and type of synthesized materials (figures 10, 16, 18, 19), GB appear as flattened vesicles (figure 19), tubular structures (figure 18), platelets (figure 16) or large oval shaped vesicles (figures 7, 9). Large oval shaped GB are involved at the site of the synthesis of pinocytotic secretory vesicles (figures 8–10). Flattened vesicles appear to be involved

at the site of the synthesis of apical electron light vesicle (Elv) (figure 19). Platelet type of GB appear to be involved at the site of the synthesis of secretory filled vacuoles (figure 16) where merocrine secretion takes place. The nuclei of epithelial cells are large, lobed and contain 4 or 5 nucleoli. The secretory epithelia exhibit different types of secretory granules and vesicles. Prominent among them are a group of dense secretory granules and vesicles (figure 9), phagosomes (figure 14), filled vacuoles (figure 16) and numerous packets of secretory materials (figures 8, 10). In a recently mated moth, the apical region exhibits numerous vacuoles which later get filled with secretory materials (figure 19). GB are intimately associated with dense cored particles. Eudocytosis of phagosomes are evident in the basal epithelia, wherein hollow uptake of solid particles in a vesicle and into a cell (figure 14). There are 4 stages of phagocytosis. Initially, the membrane appears to swell a little, enclosing some secretory materials (1st stage-EC1), which then elongates in the form of a tube (2nd stage-EC2). The third stage (EC3) involves the expansion of the tube like structure to contain probably more secretory vesicles and finally (4th stage-EC4) the enlarged part converges and closes the opening and constricts at that part. Thus results the phagosome (EC5). The basal epithelia exhibit numerous cell foldings as if to gain greater surface for synthetic activity. Dark and dense secretory granules appear to be found in the apical region (figure 10). The site of synthesis of this is granular, oval shaped and surrounded by rER. Numerous mitochondria are located around the site. Holocrine type degeneration secretion appears as the transport of these granules (figures 8, 10). But an apocrine mode of secretion occurs most frequently. Apocrine secretion involves cytolysis of the contents of the apical ends of the cell, pinching off the degenerate portion and rupturing the enclosing cell membrane. The free cell surface of the apical region accumulates the secretory material and break open the plasma membrane and empties the materials either in blebs of the cell or pinched off (figures 17, 18). Merocrine secretion appears to have small insignificant microvilli while the free cell surface of the secretory area have large microvilli and the materials pass through them. Pinocytic activity is seen in the apical region of secretory epithelia (figures 9, 12). Numerous pinocytic vesicles are being transported to the apical region as evidenced in figure 9. The secretory epithelia seemingly disintegrate continuously with the high secretory activity of the cell (figures 8, 10, 11). Luminal contents vary according to the copulatory cycle. Various secretory materials as well as eupyrene sperm bundles and apyrene individual spermatozoa are found. There are numerous dense secretory granules which appear closely associated with the spermatozoa (figures 6, 10, 11, 12). There are copious glycogen materials along with electron dense and light vesicles. Broken pieces of secretory epithelia are also found. Matrix like material appear to be suspended in the whole of the lumen duplex gland.

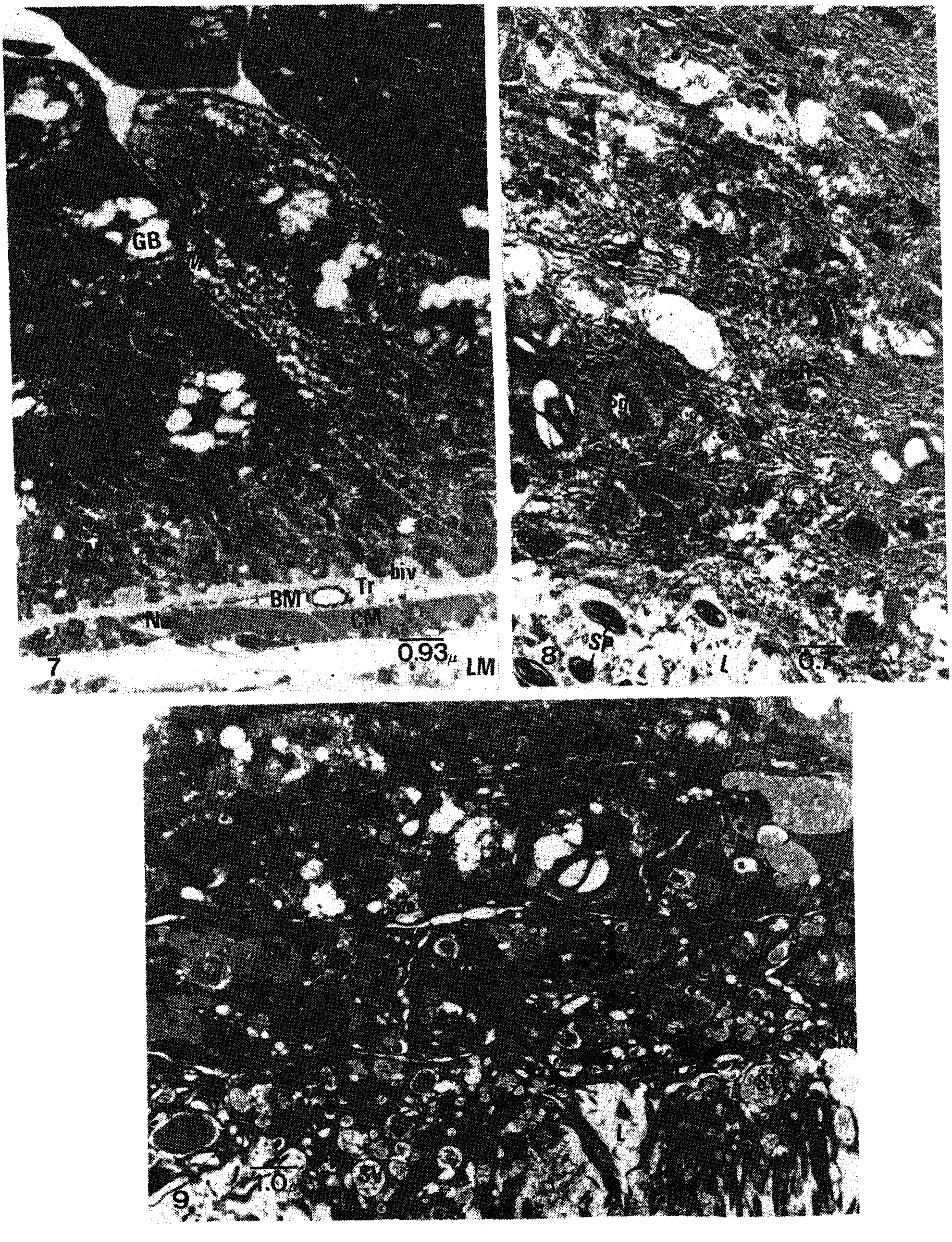
Scanning of the cross section of the duplex gland reveals that the secretory epithelium nearly fills the lumen (figure 5). The epithelial cells are tall, columnar. The cytoplasm is quite granular, the topography of the free cell surface reveals columnar epithelial cells having an even border (figure 1). The wall of the duplex appears thick and robust in the SEM. The lumen of the duplex confirms the presence of sperm bundles and individual apyrene. The sperm sheath that envelops the sperm bundle appear smooth and robust (figures 2, 3). Besides containing sperm bundles and individual sperm, the lumen also exhibit oil secretory granules (OSg) to which the sperm attaches itself. Secretory globules (Sg) appears budded off from the



Figures 1–3. **1.** SEM of cross section of the ductus ejaculatoris duplex (Duplex) of *H. armigera* showing the muscular wall, epithelial cell (EC) and the lumen (L) containing secretory globules (Sg). **2.** Section through the epithelial layer and lumen showing vividly the sperm bundles (Sb), sperm sheath (SS) and the individual spermatozoa (SP). Note the lumen appears not completely filled in. **3.** A higher magnification of the lumen with well filled oil secretory granules (OSg) to which the sperm often attaches itself. Seen are also the other secretory materials and some individual spermatozoa. Note the OSg has granular surface.



Figures 4–6. **4.** SEM showing muscle layers (ML), tall columnar epithelial cells and the secretory material (SM) filled lumen. **5.** SEM showing tall columnar epithelial cells. Note the apical degeneration of epithelium with oval shaped secretory granules filled lumen. **6.** TEM of lumen of the duplex of *H. armigera* showing spermatozoa, electron dense secretory granules (EdSg), electron light secretory granules (ElSg), abundant glycogen particles, epithelia and suspension of the matrix like materials. Note the close association of spermatozoa and dense secretory granules.



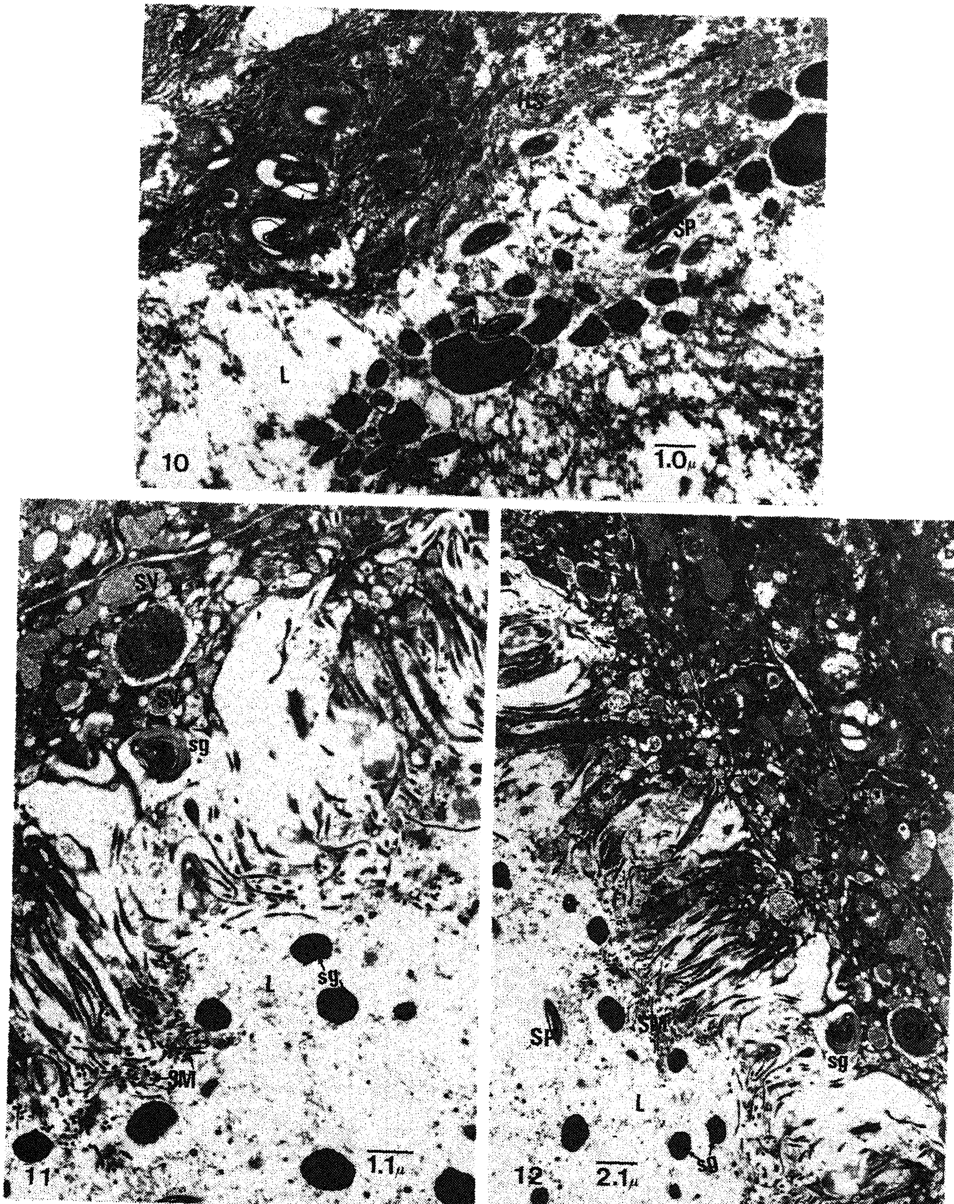
Figures 7–9.

free cell surface of the apical region of the epithelia (figure 1). They appear as oval shaped granules. At a higher secretory activity phase, degeneration of the secretory epithelia appears along with the secretion (figures 4, 5). The lumen then copiously filled with secretory materials and spermatozoa.

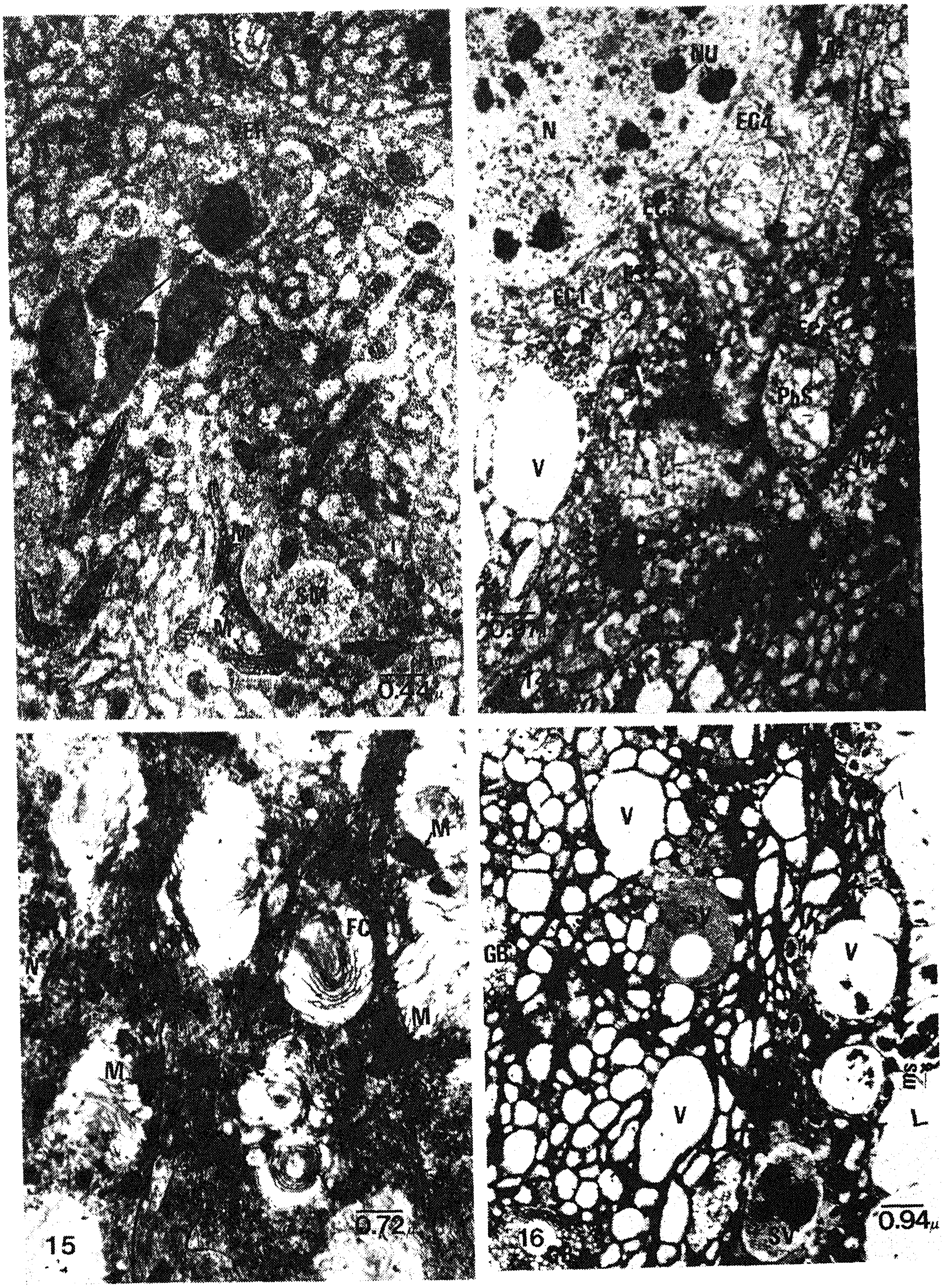
4. Discussion

Though duplex was named differently, entomologists generally stick to the term 'Duplex' (Callahan 1958). Ductus ejaculatoris duplex is also the principal storage organ for sperm in the male reproductive tract of Lepidoptera (Amaldoss 1987a, b). The duplex of *H. armigera* resembles that of most of the lepidoptera in general, particularly in morphology and in their relationship to the other glands of the male reproductive tract. Although duplex is the shortest part of the male reproductive tract, it has been reported to possess an anterior part from the point of entry of VD into the duplex (Musgrave 1937; Callahan and Chapin 1960; Callahan and Cascio 1963; Riemann and Thorson 1976b; Brits 1978; Lai-Fook 1982b). No attempt has been made to show the division, except to report an agreement with Lai-Fook (1982b) on *Calpodes* that the anterior part is shorter than the posterior part of the duplex. Although there is no mention of muscle layers in duplex wall either of *Ephestia kuhniella* by Musgrave (1937) or *Phthorimaea operculella* by Brits (1978), Callahan and Cascio (1963) reported the presence of an inner longitudinal muscle layer and an outer circular muscle layer. The opposite is the situation in *H. armigera*, circular layer being inside and longitudinal layer being outside. It is to be mentioned that the same condition has been reported by Lai-Fook (1982c) in *Calpodes ethlius* and by Riemann and Thorson (1976a, b) in *Anagasta kuhniella* but the condition of these layers are different in *H. armigera* by having a moderately thick inner robust circular layer and a thinner outer longitudinal layer. There is a thin but distinct basement membrane in *H. armigera*. Callahan and Cascio (1963) and Brits (1978) have reported a well developed basement membrane in *Heliothis zea* and *P. operculella* respectively. There is no mention of invaginations and this could possibly help in holding together the entire cell at the height of secretory activity continuous with apical degeneration (figures 8, 10, 11). The possession of a single cell type in the duplex of Lepidoptera appears to be a common feature (Riemann and Thorson 1976a, b; Lai-

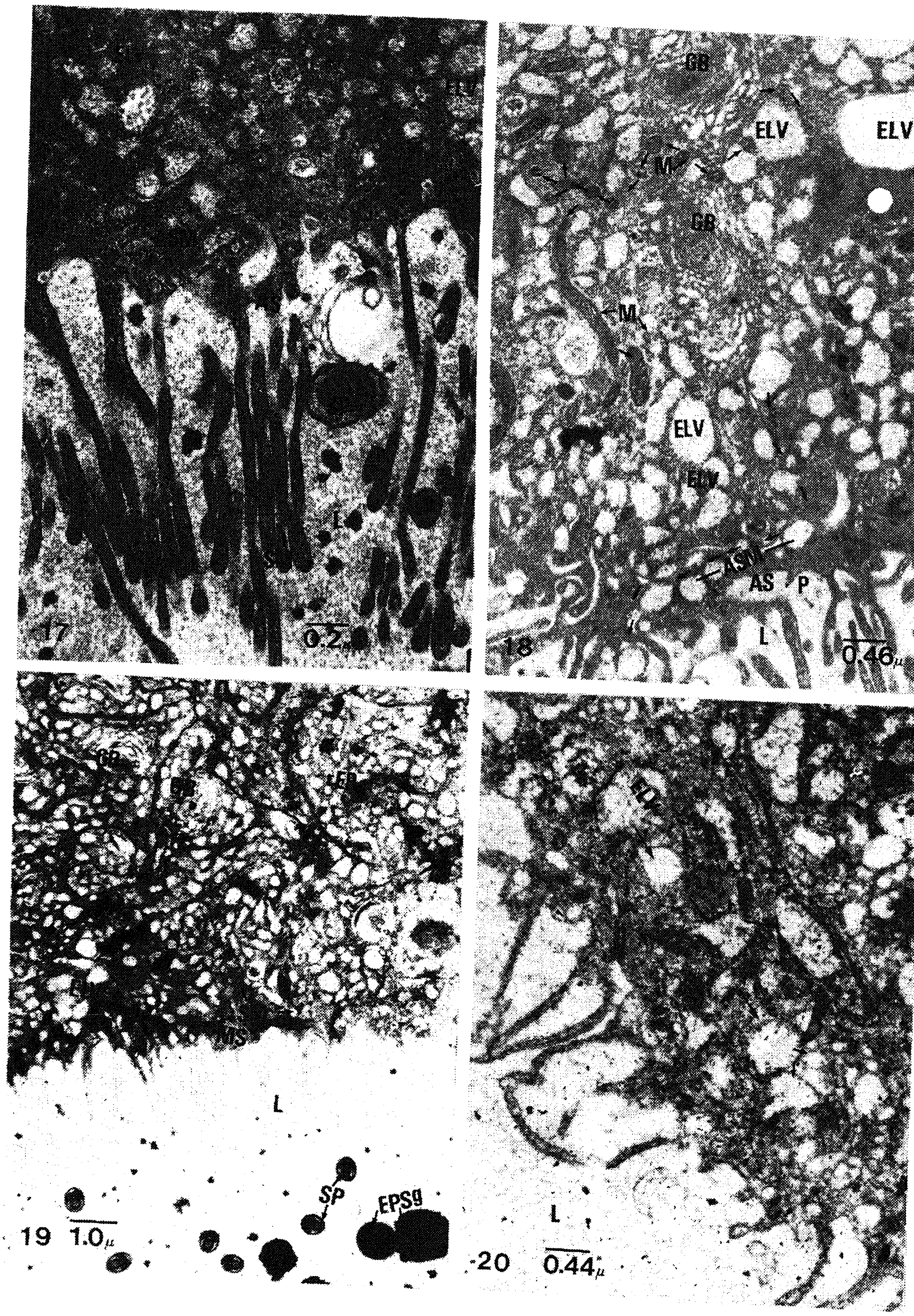
Figures 7–9. **7.** TEM of the duplex of *H. armigera* showing LM, CM, BM and basal epithelia. Note the Tr and Ne. Obvious basal invaginations biv are numerous all along the basal epithelia followed by basement membrane. Note the numerous large oval shaped GB in the basal epithelia, rER, numerous mitochondria, dense cored particles. The clear demarcation of the cell boundaries by CM enable the asynchronous activity of the cell clearly. **8.** A section through the mid and apical epithelia of duplex shows extended sacculus rER, secretory granule formation SgF stages, scattered around numerous mitochondria and large V. Note the released dark secretory granules and the awaiting spermatozoa for some sort of exchange and there it appears the degeneration of apical epithelia continuous with high secretory activity of the cell. **9.** A section through the apical region of epithelia of duplex exhibit exocytosis of pinocytic vesicles into the lumen. Note occurrence of pinocytosis. The large GB are actively synthesizing materials for pinocytic secretory materials (PSM). Note the pinocytic vesicles lining up along the free cell surface and being expelled into the lumen, are clearly seen. Endoplasmic reticulum demonstrate richly studded with ribosomes. The lumen is filled with pinocytic vesicles (SV).



Figures 10–12. **10.** TEM resembles a bit that of figure 8. A section through the apical epithelia exhibit the secretory process of dense secretory granules and transport of the same with concurrent occurrence of holocrine means of secretion (HS). Note the intact rER with the secretory process and degeneration epithelia with lysed cells. Spermatozoa closely associate with dense secretory granules. Lumen contains pieces of secretory epithelia. **11 and 12.** TEM of apical lumen regions of duplex exhibit holocrine secretion (HS) (**11**) and pinocytosis (**12**) with arrow marks. Note the lumen contain spermatozoa, dense secretory granules. Note the exocytosis of pinocytic vesicles (**12**) with arrow marks.



Figures 13–16. For caption, see p. 11.



Figures 17–20. For caption, see p. 11.

Fook 1982e). There are clear evidences of asynchronous activity of the adjacent cells within the duplex of *H. armigera* (figure 7) as also evidenced by Lai-Fook (1982e) on *Calpodes*. It is unique to see the various secretory activities, apocrine being most frequent, occasional merocrine and holocrine occurring. Both pinocytosis and phagocytosis occur. Pinocytic vesicles are exocytosized while phagosomes are endocytosized (figures 9, 14). Riemann and Thorson (1976a, b) reported the release of the secretions either through apocrine or merocrine means while Lai-Fook (1982e) reports of infrequent occurring of apocrine secretion in *Calpodes*. For Brits (1978) it is entirely merocrine, for Callahan and Cascio (1963) it is entirely apocrine and for Musgrave (1937) it is apocrine secretion coupled with epithelial proliferation. The degeneration in *H. armigera* resembles that of *H. zea* (Callahan and Cascio 1963). The disintegration of the cell is continuous with the high secretory activities of the cell. The cytoplasm containing granules and clear globules move to the free cell surface and expands the cell membrane to form a bud. By rupturing the enclosing cell membrane, the bud pinches off into the lumen. Degeneration is complete leaving only basement membrane and muscle layers intact. Riemann and Thorson (1976a, b) reported the occurrence of degeneration in aged animals. Lai-Fook (1982e) observed no occurrence of degeneration in *Calpodes*. It is important to stress that in *H. armigera* the degeneration is accompanied by high secretory activity which appears to be holocrine secretion (figures 8, 10, 11). These insects were just 5–6 days old, mated and there is accumulation of optimum secretion. The basic contents of the lumen very much resemble that of other Lepidoptera. The physiologically active condition of the duplex and physiologically inactive condition of the seminal vesicles (SV) (Amaldoss 1987a, b) require the difference of the content

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Figures 13–16. **13.** A section through epithelia of duplex which show ER studded with ribosomes and large mitochondria M. Note a group of 5 secretory granules. rER shows sacculus vesicles and with many secretory materials filled vesicles are seen. **14.** TEM of a section of secretory epithelia of duplex showing large nucleus (N) with 4 or 5 nucleolus (NU), many electron light vesicles Elv, a large vacuole V, elongated mitochondria, flattened vesiculate rER and microtubules. Note the stages of endocytosis (EC) by phagocytic activity. The final product is a phagosome (PhS). The 5 stages of phagocytic activity (EC1, EC2, EC3, EC4 and EC5) are clearly evident. **15.** A section through epithelia exhibit rER in the form of finger like configuration (FC), numerous cell foldings, numerous mitochondria. **16.** TEM of an apical region of duplex showing numerous vacuoles, platelet GB. Note the secretion filled vesicles are merocrine means of secretion (MS) expelled into the lumen. Note the secretory vesicles are accumulated at the free cell surface and the plasma membrane breaks open to expel the secretion into the lumen. **17–20.** **17.** TEM of free cell surface of duplex showing the apical means of secretion (AP) taking place. The secretory globules accumulate at the free cell surface and pinched off at the lumen. The lumen contains globules, spermatozoa and matrix materials. **18.** A section through the apical region exhibits two very large tubular GB actively synthesizing materials. Note the close association of GB with dense cored particles and numerous mitochondria. Apical secretory materials collect at the free cell surface and are expelled into the lumen. Note the secretion filled numerous electron light vesicles. **19.** A section through the apical region and lumen showing numerous cell foldings. The epithelia exhibit cell foldings, rER, numerous Elv and GB. The lumen contains SP and dense secretory granules. This appears non secretory area and the microvilli are small in size. **20.** TEM of apical region—lumen of duplex showing long irregular microvilli. Note the numerous Elv at the free cell surface. Secretory globules accumulate at the free cell surface. The lumen contains matrix.

of seminal fluid in duplex as well as in SV. The present findings go along with Brits (1978) that the duplex receives secretions from VD and ARG. Duplex is highly secretory and there appears a rich contribution from various secretory types. Though SV and duplex are functionally sperm storage organs, it is the seminal fluid of duplex that will accompany the spermatozoa and may influence the physiological condition within the duplex (figures 10, 11). As in *Spodoptera litura* (Amaldoss 1987a, b), the duplex of *H. armigera* provides conditions for physiological activity for sperm activation and motility while seminal vesicles do not. Duplex are free of sperm bundles on most of their parts. As in *A. kuhniella* (Riemann and Thorson 1976a, b) and in *Calpodes* (Lai-Fook 1982d), the eupyrene sperm bundles are positioned posteriorly while Musgrave (1937) and Callahan and Cascio (1963) describe them as positioned anteriorly. The polymorphic GB in association with mitochondria, cored particles and rER appear to be specific with respect to site of synthesis and their type of secretory materials.

Werner (1986) studying the ultrastructural changes in Golgi apparatus in the backswimmer, *Notonecta glauca* has reported 3 functional major phases of GB recognizable from their morphological characteristics. Are polymorphic GB also involved during spermiogenesis within the duplex? Whether polymorphic GB in the duplex of *H. armigera* are also functional major phases (manifesting from their morphological characteristics) requires further evidence to determine.

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Concurrent occurrence of holocrine type-degeneration along with high reproductive function in the secretory epithelia of the duplex of two Noctuid species, *Spodoptera litura* (F) and *Heliothis armigera* (H) (Lepidoptera : Noctuidae)

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Abstract. Ductus ejaculatorius duplex is the major sperm storage organ in male reproductive tract of Noctuid species. Although seminal vesicles and duplex are functionally sperm storage organs, duplex plays an important role in sperm functions on its transport from male to female. At the zenith of the reproductive function, duplex exhibits holocrine type-degeneration (secretion) concurrent with the height of secretory activity wherein the duplex secretion facilitates the transport of the spermatozoa into the female via spermatophore and contributes rich materials for spermatozoa maturity and physiological activity.

Keywords. Duplex; holocrine type-degeneration; sperm function.

1. Introduction

Over the basic plan of the male reproductive tract in Lepidoptera, there exists 4 sets of secretory tubules viz: vasa deferentia (VD), accessory reproductive glands (ARG), ductus ejaculatorius duplex (Duplex) and ductus ejaculatorius simplex (Simplex or ejaculatory duct). The varied terminology applied to duplex is very confusing, Mehta (1933) called it a paired portion of ejaculatory duct, Omura (1938) called it seminal vesicles in *Bombyx mori* (due to lack of seminal vesicles in VD), Musgrave (1937) and Stitz (1901) called it paired glands. But by and large entomologists have adhered to the term of Callahan (1958) as duplex. There has been general agreement that the duplex is formed of secretory epithelium beneath a thin layer of muscle. Apparently no one has found marked differences in the secretory cells from the different regions of the duplex (Riemann and Thorson 1976a). Lai-Fook (1982) also confirms the single cell type of duplex epithelium but he reported the occurrence of asynchronous secretory activity in the adjacent areas within the duplex. Amaldoss (1987) also confirmed a single cell type, but reports the presence of 3 layers of muscles which is unique.

Musgrave (1937) mentions the occurrence of the apocrine secretion coupled with epithelial proliferation in the duplex of *Anagasta kuhniella*. Callahan and Cascio (1963) reported the process of degeneration in the duplex of *Heliothis zea*, leaving only the basement membrane and muscle layers intact. Riemann and Thorson (1976a) reported the same process in *Anagasta kuhniella* and this occurred in both the epithelium and the muscle layers in aged animals. Lai-Fook (1982) fail to observe this process even in animals aged 14 and 17 days. Amaldoss (1987) reported in *Spodoptera litura* and in *Heliothis armigera* (unpublished data) the process of degeneration while describing their ultrastructure. In the light of their

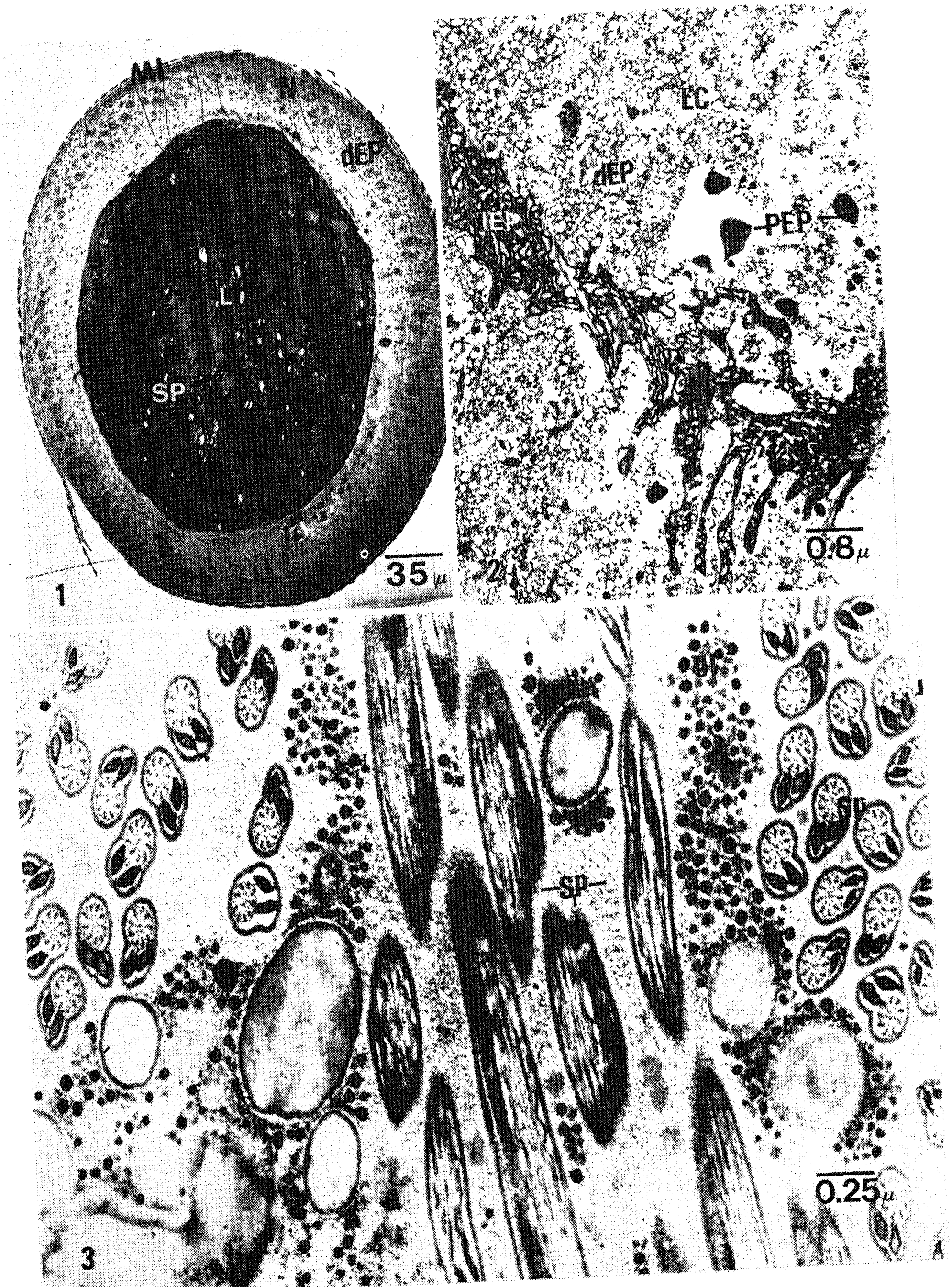
findings, it is intended to trace the phenomenon of degeneration and its implications in the reproductive function of the two Noctuid species *S. litura* and *H. armigera*.

2. Materials and methods

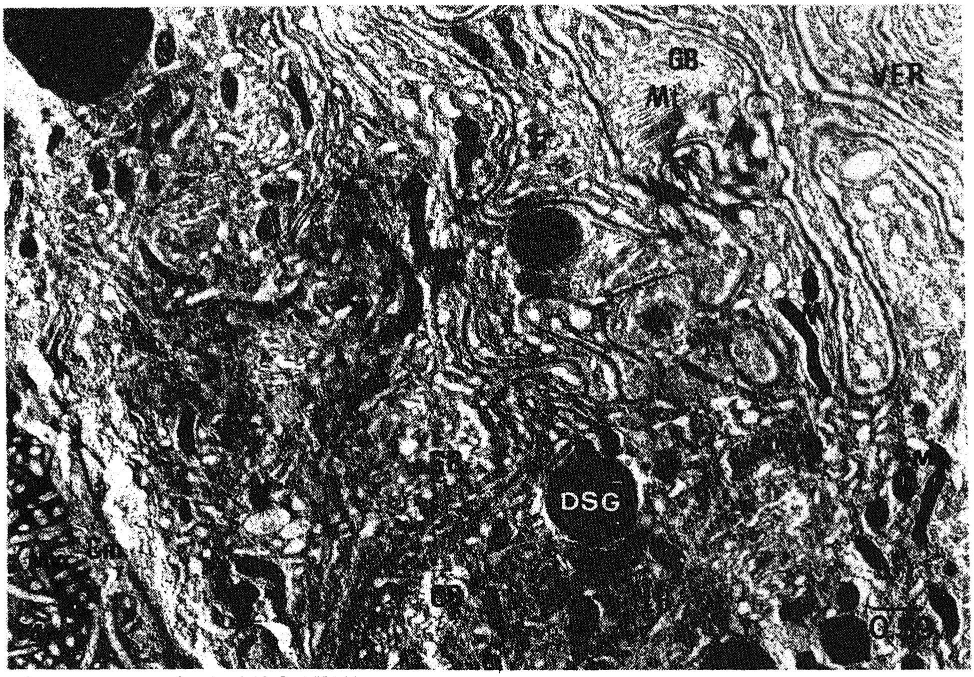
The rearing of animals was done as previously described (Amaldoss 1987), and 4–5 day old mated males were chosen for these experiments. The male reproductive tract was dissected out in Weevers (1966) lepidopteran saline and duplex glands were removed carefully and fixed in 2% glutaraldehyde-with 0·1 M phosphate buffer (pH 7·4) for 3 h. The tissue were washed thoroughly in the same buffer and post fixed in osmium tetroxide (OsO4) for 1·5 h and washed again thoroughly in the same buffer. Then the tissues were dehydrated in a graded alcohol series and embedded in spur Epon 812. Thin sections were made in a C Reichert Austria OM3 Ultramicrotome. The thin sections were double stained in urayl acetate for 5 min and lead acetate for 50 s and viewed under JEOL, JEM 100S transmission electron microscope and micrographs were taken.

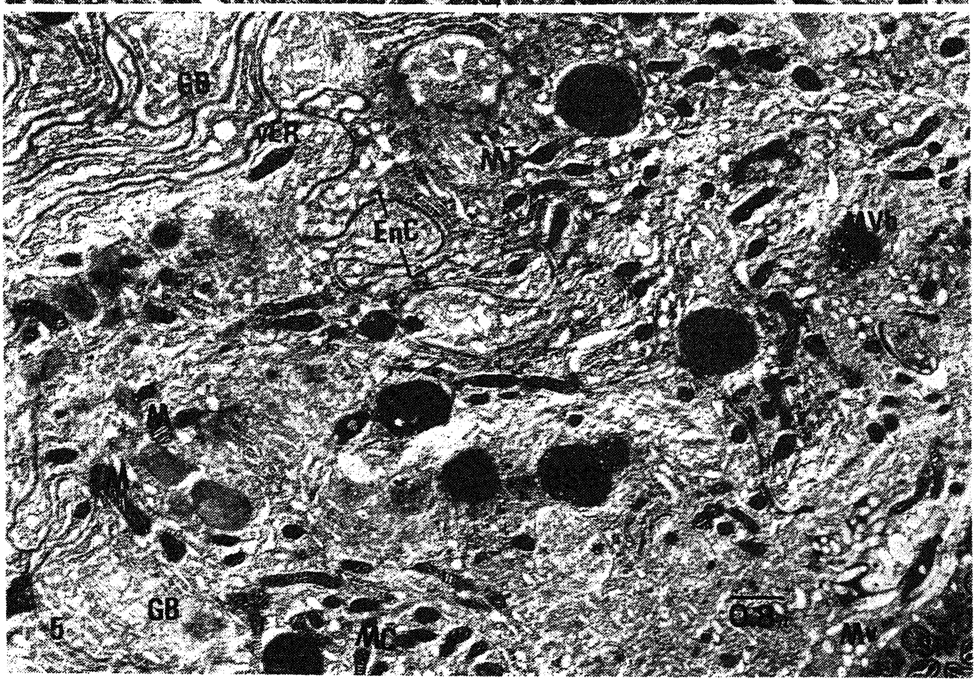
3. Results

Both *H. armigera* and *S. litura* like other Lepidoptera exhibit a single cell type but with asynchronous secretory activity in the adjacent cell within the same duplex glands (Amaldoss 1987 and personal communication). *S. litura* exhibits 3 layers of muscle, two of which are inner circular muscle layers and *H. armigera* exhibit only two layers of muscle one of which is an inner circular muscle layer (figure 6). Duplex of both measure 7 mm in length and 1 mm in diameter. Virgin duplex of 1 or 2 day after eclosion show very little secretory activity. The lumen contains a few dense secretory granules and globules. The lumen is basically empty. The tall columnar epithelial cells appear as if to close the lumen. On the fifth day after eclosion, optimum secretion occurs with eupyrene sperm bundles and individual apyrene sperm (figure 1). The muscle layers, distinct basement membrane and the tall columnar epithelia make the wall of duplex appear thick and robust but as the entry of spermatozoa make their way into the duplex via the VD from the testes and the secretions accumulate, the wall of duplex is pulled and pushed to give more space for the content of the lumen. Thus the wall of duplex becomes thin on the fifth day or after mating. *Spodoptera litura* exhibit apocrine mode of secretion frequently (figures 4–6) which involves cytolysis of the contents of apical ends of the cell, pinching off of the degenerate portion and rupturing of the enclosing cell membrane (CM). Occasionally merocrine means of secretion takes place. Certainly this mode of secretion is infrequent. Holocrine type of degeneration of epithelia is evident in the secretory epithelia. But this appears not to happen in virgin males but only in mated males. Both in *H. armigera* and *S. litura*, the apocrine secretion is prominent with accumulation of pinocytotic vesicles and numerous phagosomes. Phagosomes are endocytosized and pinocytic vesicles are exocytosized. The polymorphic Golgi bodies (GB) and multishaped rough endoplasmic reticulum (rER) concentrate around their site of synthesis and the type of secretory materials. The high secretory activity is accompanied by degeneration of the apical region of



Figures 1–3. 1. Thick section on a cross section of ductus ejaculatorius duplex of *S. litura* showing the external thick muscle layer (ML), basally located nuclei (N), the sperm (SP) and seminal fluid filled lumen (L). Note the degenerating epithelia (dEP). **2.** Thin section from a cross section of degenerating epithelia (dEP) showing lysed cells (Lc), segregated piece of epithelia (PEP), left over intact epithelia (IEP). **3.** Lumen showing the spermatozoa (SP) in cross and longitudinal angles, glycogen particles (gl), large unknown granules.





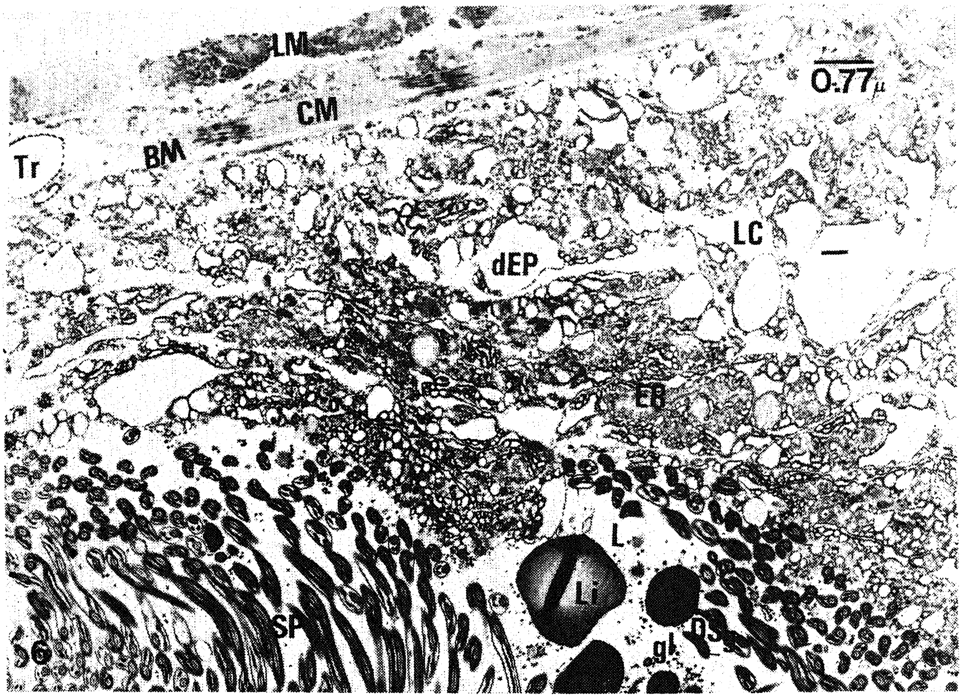
Figures 4 and 5. **4.** Epithelia of duplex at the height of secretory activity showing numerous Golgi bodies (GB), rough endoplasmic reticulum (rER), large number of microtubules (Mt), well distributed mitochondria (M), dark dense secretory granules (DSg) and numerous secretory vesicles. Note the cell foldings and the pinocytotic vesicles appear to be gathering at the free cell surface (FCS) and released into the lumen breaking open the cell membrane (CM) through the micro villi (MV) (at left hand corner). **5.** This electronmicrograph like the preceding one, exhibit endocytosis of secretory material (EnC). Note the multivesiculate body (MVb). At the right hand corner note the free cell surface with large number of pinocytotic vesicles and the lumen containing few spermatozoa.

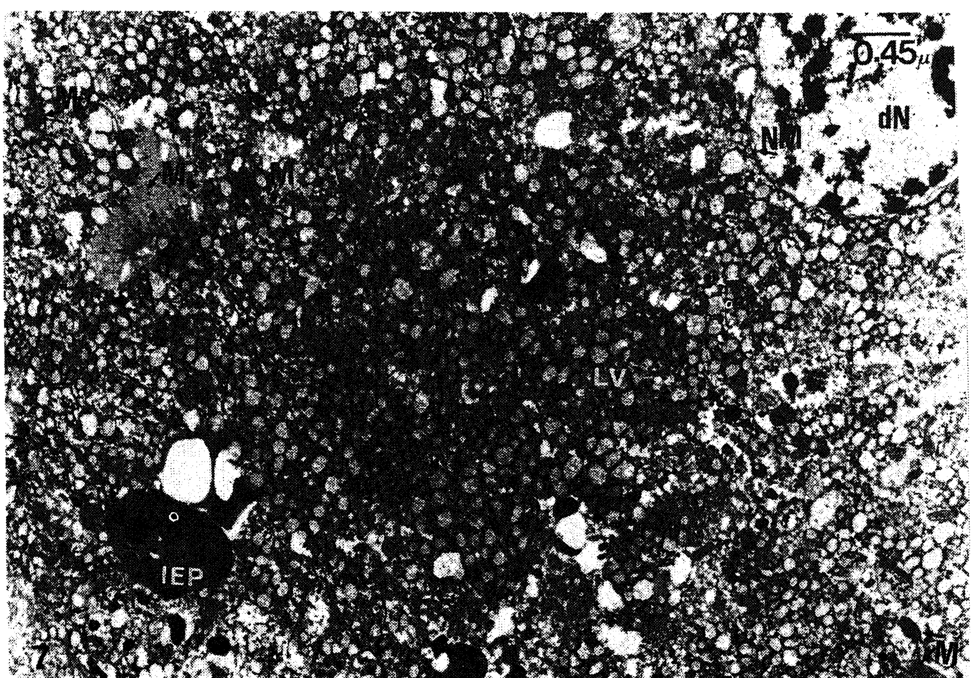
secretory epithelia wherein the apocrine secretory materials and the degenerative apical cells descend together (*H. armigera*).

The process of degeneration begins with apical proliferation of epithelia. During the process of degeneration, cytoplasm, containing granules travels to the free cell surface and expands the cell wall to form a bud. This bud constricts off at the neck of the cell. The liberated bud is thus expelled into the lumen as a free entity (*H. armigera* and *S. litura*). This process appears as the result of frequent and heavy apocrine secretion. Some areas of secretory cell show exocytosis of secretory granules wherein lysis of cells takes place and the contents in a cell are washed away into the lumen. It appears that this process accompanies the transport of already produced dense secretory granules (*H. armigera*) into the lumen. During this process the spermatozoa migrate deep into the lysed basal secretory epithelia (figure 6). Other areas where pinocytosis takes place, pinocytotic vesicles are endocytosized into the lumen (figures 4, 5). This is also seemingly accompanied by a holocrine type of degeneration in (*H. armigera*) and possibly play a role in transporting the secretory materials into the lumen. Figures 6 and 7 depict the degeneration of the entire epithelium leaving only muscle layers and basement membrane intact. The cells are getting lysed, part of the rER undergoing autolysis and the cell boundaries are still recognizable (figure 6). Numerous lysosome and resultant vacuoles from the cell lysis are also seen. The degenerate nucleus still exhibits the intact double nuclear membrane and the nuclear materials within the nucleus appear to have been also affected (figure 7). The entire epithelia reveals left-over intact pieces of epithelia, a number of mitochondria, lysosomes, dense cored particles or vesicles, clusters of glycogen and scattered microtubules. The spermatozoa appear to reach out into the entire lysed materials within the degenerated epithelium (figure 6). This has been the observation almost among all the holocrine type degenerative epithelia of duplex both in *H. armigera* and *S. litura*. There is still another area of holocrine type degeneration which show the degeneration of the rER, (figure 8, 9). This process of degeneration of rER involves the enlarging of rER making a bend and swelling on one side. Inside the swelled part, there appears to be a kind of a enzymatic break down of materials. This leads to the opening of the swollen part, and ribosomes are liberated as free ribosomes or as accumulated ribosomes. The membranous or tubular part of the rER then appears to dissolve. Now the resultant two materials morphologically appear different. Free ribosomes (liberated and accumulated ribosomes) appear as electron light little particles, while the dissolved tubular part rER appears as electron dense large particles. All these particles appear to move to the free cell surface and hang around the plasma membrane. The plasma membrane then, at the free cell surface, in its turn appears to be distended. Materials or the rER particles appear to pass through the plasma membrane by a kind of diffusion (figures 10, 11, 12).

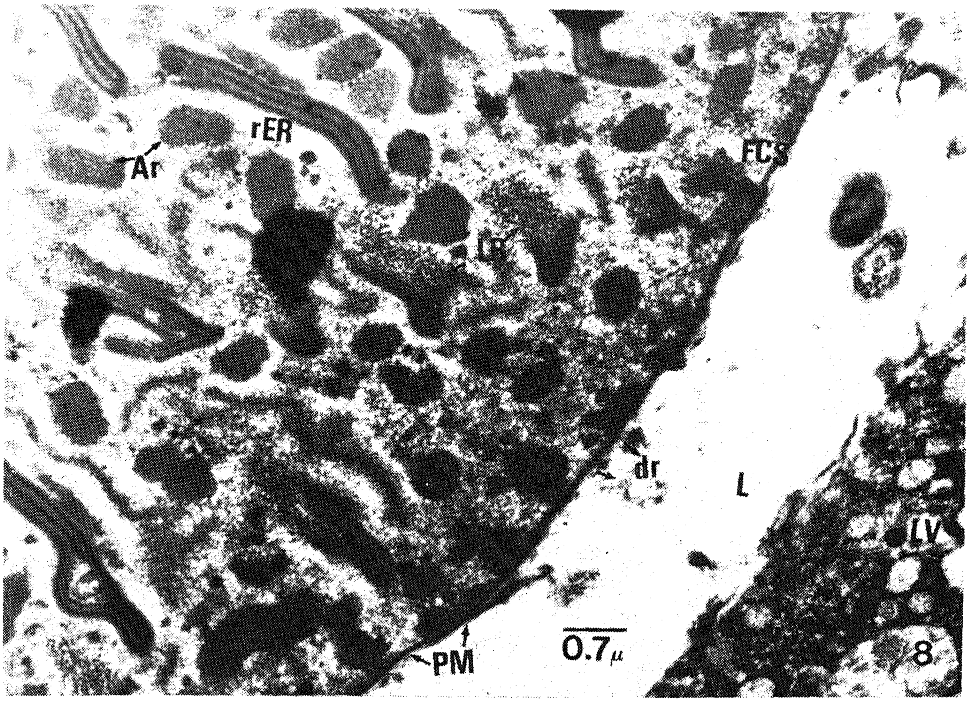
4. Discussion

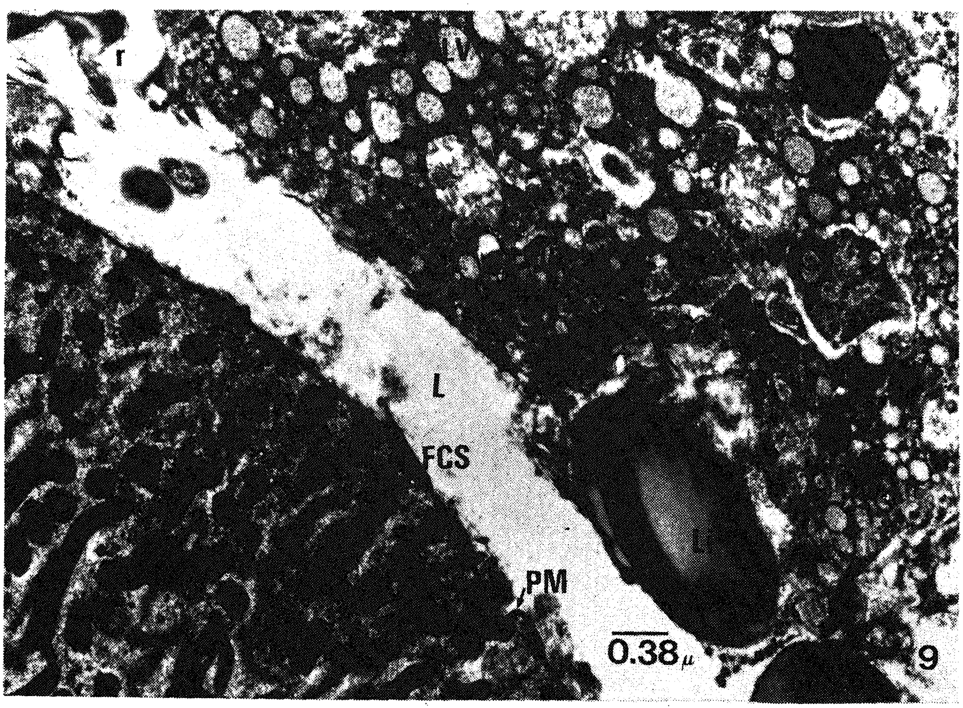
Seminal vesicles and ductus ejaculatorius duplex are the two major sperm storage organs in most of the male Lepidoptera except *Bombyx mori*. There are only a pair of duplex which are termed seminal vesicles in *Bombyx mori* (Omura 1938) because they lack the typical enlarged portions of VD to form the seminal vesicles. It is generally acknowledged that sperm periodically descend from the testes to VD, SV and finally to duplex (Riemann and Thorson 1976b; La Chance *et al* 1977). The quantity of sperm in the seminal vesicles is influenced by mating. The emptying of



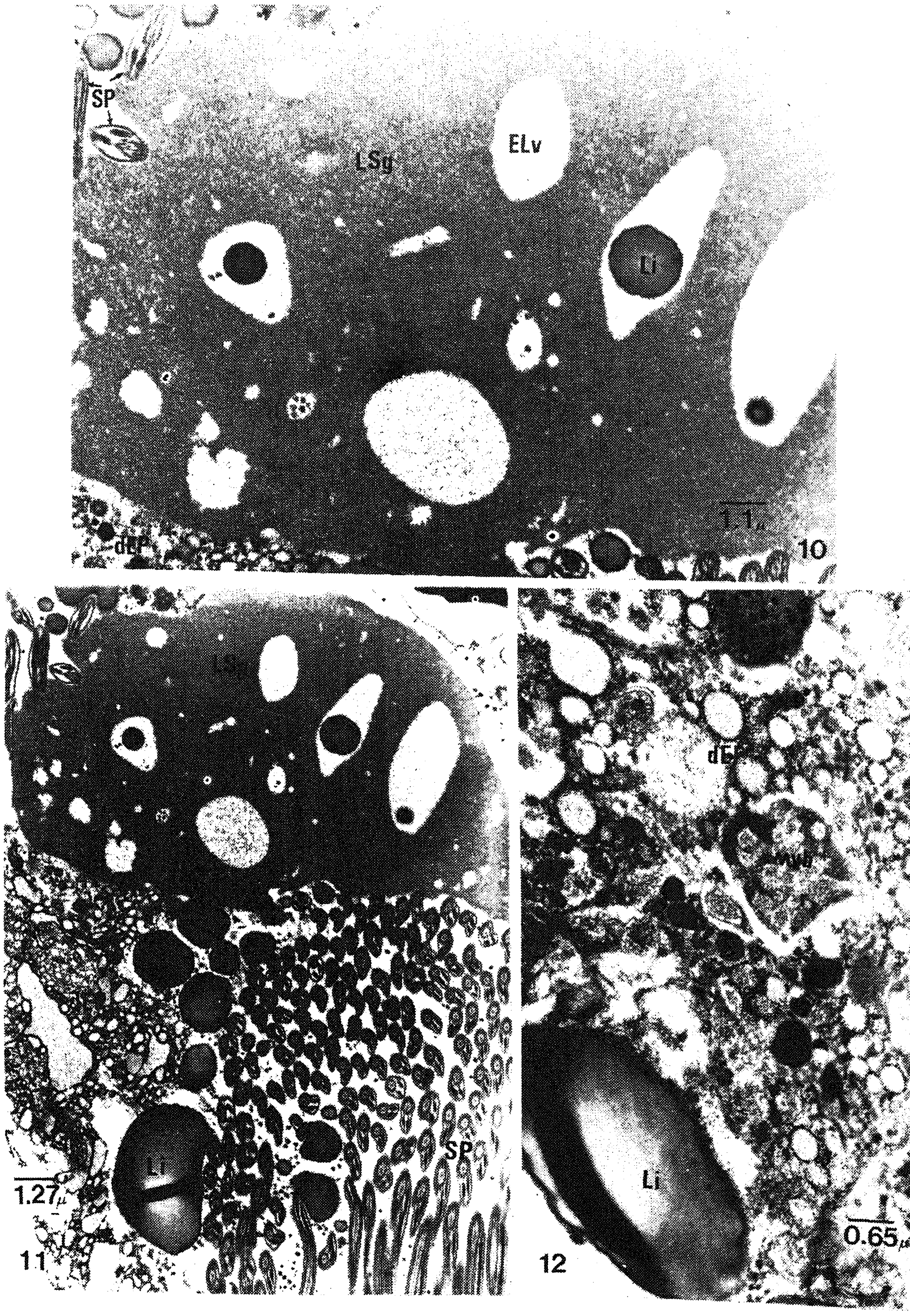


Figures 6 and 7. **6.** Holocrine type degenerating epithelia of duplex showing the intact 3 muscle layers (LM, CM), the basement membrane (BM) and the trachea (Tr). Note the spermatozoa appear to migrate very deep into the basal degenerated epithelia (dEP). Also seen are the dense secretory granules (DSg), lipids (Li), lysed cell (Lc) and glycogen particles (gl). **7.** Holocrine type degenerated cell. Note the cell is completely lysed. Note also the mitochondia (M), degenerating nucleus (dN) with intact nuclear membrane (NM) and intact epithelial pieces (IEP) and lysed vesicles (LV).





Figures 8 and 9. **8.** Degenerating rough endoplasmic reticulum (rER) and the process of liberating of ribosomes (LR) and the accumulated ribosomes (AR), migration free ribosomes to the free cell surface (FCS) and diffusion. **9.** Through the apical region and lumen of holocrine type degeneration cell showing degeneration (dEP) of rER and the lumen with lysed and other materials. Note two large lipid droplets.



Figures 10–12. 10 and 11. Lumen of holocrine type-degenerating epithelia with unknown large secretory granule (Lsg) which appears to be multivesiculate body by havi numerous vesicles. Note the spermatozoa (SP), degenerated epithelial pieces (dEP), a lipid droplets. **12.** Lumen of holocrine type degeneration cell with multivesiculate bodi large lipid droplets.

duplex during copulation results in most of the sperm being released from the SV (Riemann and Thorson 1976b). Seminal vesicles exhibit a physiologically inactive condition while duplex exhibits a physiologically active condition for sperm activation (Amaldoss 1987). The duplex of virgin males show less secretory activity both in *S. litura* and *H. armigera* and the lumen contain few discrete secretory granules. At the height of reproductive activity, the duplex demonstrates asynchronous activity in the adjacent cells within it. As is the case in *A. kuhniella* (Riemann and Thorson 1976a, b), duplex of *S. litura* and *H. armigera* secrete profusely after mating. Apocrine was the most frequent mode of secretion. Various forms of rER, polymorphic Golgi apparatus, many lysosomes, cored vesicles, numerous dense granules, microtubules were all in abundance in the process of secretion. Pinocytotic vesicles and phagosomes are numerous. Endocytosis of phagosomes and exocytosis of pinocytic vesicles are evident. Similar conditions were reported in *A. kuhniella* (Riemann and Thorson 1976a). All these indicated high secretory activity in the duplex of 4–5 days old males after mating. Musgrave (1937) reported occurrence of apocrine secretion with epithelial proliferation as also Callahan and Cascio (1963) and Riemann and Thorson (1976b). Callahan and Cascio (1963) reported exclusive occurrence of apocrine while Riemann and Thorson (1976b) reported frequently apocrine, occasionally merocrine. Duplex of *S. litura* and *H. armigera* also exhibit apocrine frequently or even dominantly while merocrine is rare. Lai-Fook (1982) reports the occurrence of merocrine and infrequent apocrine. For Brits (1978) it was entirely merocrine.

Yung-Tai (1929) and Henson (1930) contended that the process of disintegration occurs following active periods of secretion in the ventricular epithelium. Callahan and Cascio (1963) reported that the degeneration of the cells of duplex in *H. zea* is very similar to the degeneration of the digestive cells as described by Snodgrass (1935), Folsom and Welles (1906), Needham (1897) and Yung-Tai (1929) except that there are no regenerative cells present. Snodgrass also reported that there has been a general view that this process was considered as a method for rapid discharge of secretory products. Although Yung-Tai (1929) challenged this view, Snodgrass asserted that conclusions must be justified only by studying the physiological stand point of this process. It has become apparent that the seminal environment of the ejaculated sperm cells represents a specialised chemical medium which has no counterpart among the other secretions elaborated by the male organism (Murray *et al* 1962).

Secretions elaborate throughout the reproductive life of the male. At the zenith of the reproductive life when there are chances to copulate and transfer semen successfully to the female, holocrine type of degeneration occurs. Lai-Fook (1982) not only could not spot this type of degeneration but also failed completely to observe this phenomenon in 14 and 17 day old animals. Callahan and Cascio (1963) reported in *H. zea* that as the secretion elaborated throughout the reproductive life of the male, degeneration also takes place which continues till old age, only retaining basement membrane and muscle layers. This is significantly different in *H. armigera* and *S. litura* 4 or 5 day old mated males which exhibited holocrine type degeneration with only basement membrane and muscle layers intact. It appears this occurrence has more to do with the height of the reproductive activity and function than with aging. What occurred at the height of the reproductive function cannot be merely on aging process, but could be a culmination point of the

reproductive function. Since mating empties the content of the lumen of duplex (Riemann and Thorson 1976b), the holocrine type-degeneration appears to prepare the duplex for subsequent matings. So this type of degeneration plays a functional role in the further reproductive functions of the mated males which is vital for reproductive success. Under this evaluation, holocrine type-degeneration may show some evolutionary significance. Callahan and Cascio (1963) reported that disintegration is continuous with the secretory activities of the cells. Our observation is the concurrent occurrence of holocrine type degeneration at the height of secretory activities of the cells as observed in the duplex of both *S. litura* and *H. armigera*. The resultant degenerated cellular substances appear to show affinity with the spermatozoa, since the spermatozoa appear to enter into the basal region of the holocrine type-degenerative cell in search of those materials. In other places the degeneration also appears to join the transport of the dark dense secretory granules along with the lysed materials into the lumen. This could possibly contribute to the functional anatomical aspect of the duplex, by changing the condition of duplex physiologically ideal for sperm reproductive function. Sperm suspension, distribution, nutrition, and transport are possibly contributed from the holocrine type-degenerative materials. Since holocrine type degeneration takes place in the reproductive life of the male after mating, the resultant accumulation of secretory materials plays a functional role for the spermatozoa of duplex to remain physiologically active for further reproductive functions when remating takes place.

The holocrine type degeneration and its implications within the sperm storage organ duplex awaits further biochemical analysis of the content of the same.

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Effect of cold storage of newly hatched larvae on survival rate, growth and egg production in silkworm *Bombyx mori* L.

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Abstract. Effects of refrigeration ($5 \pm 1°C$) of newly hatched silkworm larvae on survival rate, growth and egg production were studied in two multivoltine races, Pure Mysore and Hosa Mysore. When refrigerated for 10 days, moth emergence (males: 83·3% vs 89·4% and females: 92% vs 90·2%) and fecundity (465·5 vs 458·3) did not differ significantly from the control. Reductions in cocoon weight by 4% (1·323 g vs 1·271 g) and 10·7% (1·323 g vs 1·181 g) were caused by 5 and 10 days of refrigeration. Marked increase in early stage (I and II instar), loss of larvae (12·9% vs 48·8%), decrease in effective rate of rearing (77·1% vs 33·5%), pupation rate (92·4% vs 65%) and reproductive rate (137·7 vs 36·9) were observed, when refrigerated for 10 days. Results on reproductive rate indicated one day's refrigeration as safe period in Hosa Mysore but not in Pure Mysore. In December season, both races showed higher tolerance and possibility of safe refrigeration upto 2 days.

Keywords. *Bombyx mori*; early stage loss; effective rate of rearing; pupation rate; reproductive rate; egg production.

1. Introduction

Refrigeration of cocoon or pupa, moth, egg and larva at one or more stages are followed to synchronise silkworm rearing with the availability of mulberry leaf. In silkworm seed production, refrigeration of seed cocoon and moth are inevitable to adjust adult emergence or effect mating of desired parents. Usually, refrigeration of cocoon is limited to 1 to 2 days, though the recommendations are up to 3 days for female and 7 days for male pupa (Tazima 1962; Jolly 1983). In case of moths, it is limited to 3 days in female and 7 days in male (Ullal and Narasimhanna 1978; Jolly 1983). The efficiency of mating of male moths was improved, when kept at $5 \pm 2°C$ (Subramanyam 1982). Bivoltine eggs, characterised by hibernation, are refrigerated at 2·5°–7·5°C for different period to activate them to hatch at the desired time (Mizuno 1920; Watanabe 1931; Katsukake 1952). The non-hibernating multivoltine eggs can be cold stored on second day at 5°–7°C to postpone hatching up to 24 days (Dutta *et al* 1972). Eggs at blue body stage can be refrigerated for 2 to 3 days at 5°C to delay hatching (Tanaka 1964). Refrigeration of newly hatched silkworm larvae is also not uncommon, especially when hatching is irregular and a single brushing is desired. However, unlike other stages of pupa, moth and egg, information on refrigeration of hatched larva and subsequent rearing performances are limited though not totally lacking (Jolly 1958, 1983; Tazima 1962; Tanaka 1964). The present study is carried out to understand more about the effect of refrigeration of newly hatched silkworm larvae on their rearing performances and reproductive rate.

2. Materials and methods

Two multivoltine races, Pure Mysore (PM) and Hosa Mysore (HM) were used. Newly hatched larvae refrigerated for 1–10 days and the control without refrigeration formed different treatments. Refrigeration was done at $5 \pm 1°C$ and RH $75 \pm 5\%$, in a commercial silkworm seed cold storage. Hatched larvae, continuously for 10 days from freshly prepared layings for each day, were refrigerated and were released on a single day to facilitate brushing and rearing at a time. Each treatment consisted of 3 cellular replications. Rearing was conducted as per standard recommendations (Krishnaswami *et al* 1973). Test was repeated in 3 distinct seasons [June–July 1986 (S_1), August–September 1986 (S_2) and December 1986 (S_3)], to concur the results.

Different parameters studied were lost during early instar (I and II) rearing (early stage loss = ESL), effective rate of rearing (ERR), cocoon weight, pupation rate (PR), female and male moth emergence, fecundity and reproductive rate (RR). The definition of these terms and calculations are interpretable as given in standard sericulture text books (Krishnaswami *et al* 1973; Ullal and Narasimhanna 1978; Narasimhanna 1988) and related publications (Krishnaswami 1978, 1979; Benchamin and Krishnaswami 1981a, b). Analysis of variance (3 way factorial) was carried out for interpretation of results.

3. Results and discussion

3.1 *Survival rate*

In the present study, all treatments recorded significant increase in ESL of worms compared to control and it increased with the increased days of refrigeration (table 1). The ERR was reduced significantly in all treatments, when refrigerated for 10 days. ESL increased from 12·9–48·8% and ERR decreased from 77·1–33·5%. Inability to feed and moult normally, resulting in irregular growth, were characteristic of larvae refrigerated for more than 5 days in both the test races. Those larvae that survived in these treatments were also prone to viral and bacterial diseases in later instars. Starved larvae in silkworm *Bombyx mori* are more susceptible to flacherie (bacterial disease) and grasserie (viral disease) (Samson *et al* 1981). In the present study, even refrigeration for 1 day caused 18·6% increase in ESL (12·9% vs 15·3%) and 7·1% reduction in ERR (77·1% vs 71·6%).

Significant races × treatments and races × seasons interaction (table 6) showed specific advantages of HM race over PM and S_3 season over other seasons. For example ESL in 1 day refrigerated batch and ERR up to 2 days refrigeration in HM, were not significantly different from the control. In PM, increase in ESL and reduction in ERR were significant in all treatments (table 2). In respect of ESL and ERR, 2 days refrigeration was found safe in S_3 season.

3.2 *Growth*

Cocoon weight is directly correlated to the larval body weight. It reduced significantly in all treatments when compared to control, but did not vary significantly

Table 1. Effect of refrigeration ($5 \pm 1°C$) of newly hatched larvae on various characters studied (mean of 3 seasons).

Refrigeration (days)	Larval loss in early instars (%)	Effective rate of rearing (%)	Cocoon weight (g)	Pupation rate (%)	Female emergence (%)	Male emergence (%)	Fecundity (No)	Reproductive rate (No)
Control	12·9 (17·88)	77·1 (61·89)	1·323	92·4 (73·97)	92·0 (73·80)	88·3 (70·79)	465·5	137·7
1	15·3 (22·46)	71·6 (58·06)	1·281	89·2 (71·40)	90·4 (72·14)	90·2 (71·85)	460·1	121·1
2	17·9 (24·78)	68·0 (54·85)	1·285	87·7 (70·92)	89·9 (71·65)	87·3 (69·79)	464·6	112·7
3	25·0 (28·45)	59·7 (52·39)	1·277	83·7 (68·77)	89·4 (71·01)	88·0 (70·32)	459·5	102·6
4	27·3 (32·62)	58·5 (48·29)	1·261	85·5 (68·38)	89·8 (71·49)	86·0 (69·21)	463·0	91·8
5	31·1 (33·49)	53·4 (47·37)	1·271	80·4 (65·26)	89·8 (71·49)	88·5 (71·51)	463·5	83·6
6	33·2 (34·61)	51·3 (44·88)	1·228	80·5 (64·97)	90·3 (71·98)	88·4 (70·54)	458·5	80·2
7	33·4 (35·73)	45·3 (41·78)	1·237	72·3 (60·77)	90·9 (72·56)	88·2 (71·28)	456·5	63·0
8	41·2 (40·68)	41·2 (39·43)	1·235	76·3 (59·06)	90·5 (71·31)	86·6 (69·61)	460·0	54·9
9	50·0 (44·96)	37·6 (37·48)	1·245	73·3 (58·58)	92·0 (73·72)	87·9 (70·64)	461·3	47·5
10	48·8 (45·96)	33·5 (34·67)	1·181	65·0 (52·62)	90·2 (72·09)	89·4 (71·98)	458·3	36·9
SE	±0·788	±0·745	±0·009	±0·898	±0·640	NS	NS	±2·44
CD at 5%	2·21	2·09	0·026	2·51	1·79			6·9

Numbers in parentheses are transformed values.

Table 2. Early stage loss (%) and ERR (%) influenced by refrigeration (5 ± 1°C) of newly hatched larvae.

Refrigeration (days)	Early stage loss (%)*		ERR (%)*	
	PM	HM	PM	HM
Control	17·57	18·20	63·23	60·56
1	26·21	18·71	55·79	60·33
2	26·59	22·98	51·60	58·11
3	29·61	28·28	50·69	54·08
4	32·71	32·54	47·49	49·09
5	33·62	33·56	46·83	47·91
6	35·01	34·20	42·18	47·59
7	35·54	35·92	39·92	43·65
8	39·63	41·72	40·07	38·78
9	42·85	47·06	39·94	35·03
10	43·72	48·14	36·82	32·52
SE ±	1·114		1·054	
CD at 5%	3·12		2·95	

*Transformed values.

Table 3. Cocoon weight (g) influenced by refrigeration (5 ± 1°C) of newly hatched larvae.

Refrigeration (days)	Races		Seasons		
	PM	HM	S_1	S_2	S_3
Control	1·133	1·513	1·451	1·103	1·416
1	1·055	1·506	1·410	1·063	1·369
2	1·056	1·514	1·386	1·107	1·362
3	1·002	1·552	1·419	1·052	1·360
4	1·032	1·489	1·417	1·025	1·341
5	1·005	1·537	1·444	1·032	1·336
6	0·965	1·491	1·308	1·048	1·329
7	0·969	1·504	1·339	1·045	1·326
8	0·970	1·501	1·351	1·041	1·314
9	0·984	1·505	1·370	1·038	1·320
10	0·930	1·436	1·266	0·964	1·320
SE ±	0·013			0·016	
CD at 5%	0·037			0·045	

among the first 5 days and also among 6–9 days of refrigeration (table 1). Results indicated that growth in those larvae that survived and formed cocoons, was more or less same and it was affected at lesser degree compared to ESL and ERR. This is more clear in seasons S_2 and S_3 over S_1 and in race HM over PM, where cocoon weight was not affected among treatments of 1–9 days refrigeration (table 3).

3.3 *Egg production*

Pupation rate, female and male emergence and fecundity were the characters studied, as factors contributing to egg production. The reproductive rate, expressed

as multiplication rate in earlier studies (Benchamin and Krishnaswami 1981a, b), was also assessed as an index to compare the egg production efficiency in different treatments. Among these, male moth emergence and fecundity were not affected significantly by refrigeration (table 1). Female moth emergence was also not influenced, as the differences among treatments and between many treatments and control were not significant. PR differed significantly in all treatments compared to control and it steadily decreased with the increase in refrigeration days, from 92·4% in control to 65% in 10 days refrigeration. Race HM showed better tolerance to the treatment, since PR remained not significantly ($P<0{\cdot}01$) different from the control,

Table 4. Pupation rate (%)* influenced by refrigeration (5 ± 1°C) of newly hatched larvae.

Refrigeration (days)	Races		Seasons		
	PM	HM	S_1	S_2	S_3
Control	74·83	73·10	74·25	76·06	71·59
1	70·26	72·54	72·73	71·01	70·42
2	69·29	72·55	67·39	73·94	71·41
3	64·49	73·04	67·50	69·69	69·11
4	65·96	70·80	65·27	70·34	69·63
5	62·20	68·30	61·77	68·57	65·43
6	60·59	69·37	59·85	67·92	67·17
7	56·56	61·56	50·51	62·22	64·45
8	59·81	61·78	51·31	67·23	63·85
9	58·56	58·59	45·87	65·59	64·28
10	53·14	52·11	39·99	58·99	58·88
SE ±	1·269			1·555	
CD at 5%	3·56			4·35	

*Transformed values.

Table 5. Reproductive rate influenced by refrigeration (5 ± 1°C) of newly hatched larvae.

Refrigeration (days)	PM				HM			
	Seasons				Seasons			
	S_1	S_2	S_3	Mean	S_1	S_2	S_3	Mean
Control	113·62	125·63	126·80^a	122·02	149·86^b	139·08^c	171·29^d	153·41^e
1	77·17	98·97	111·04^a	95·73	149·63^b	128·96^c	160·86^d	146·48^e
2	56·28	76·65	116·36^a	83·09	136·27^b	133·31^c	157·20^d	142·26
3	50·86	76·31	105·74	77·64	123·55	106·76	152·21	127·51
4	52·67	71·40	101·64	75·24	106·27	94·23	124·66	108·33
5	39·10	73·25	89·52	67·29	103·68	87·11	108·92	99·90
6	35·59	41·45	93·94	56·99	104·77	89·56	115·66	103·33
7	28·76	31·04	82·77	47·53	65·87	68·73	100·67	78·44
8	27·83	30·30	75·96	44·69	59·21	36·85	99·38	65·15
9	29·01	35·05	68·16	44·07	39·07	24·45	88·93	50·82
10	16·63	26·07	60·88	34·53	20·99	21·08	75·95	39·34
SE ±		5·99		3·46		5·99		3·46
CD at 5%		16·80		9·70		16·80		9·70

Treatments indicated by same letter are not significantly different from control.

Table 6. Analysis of variance.

Source of variation	df	Larval loss in early instars	Effective rate of rearing	Cocoon weight	Pupation rate	Female emer- gence	Male emer- gence	Fecundity	Rep tiv
Race (R)	1	NS	**	**	**	**	NS	**	
Seasons (S)	2	**	**	**	**	NS	NS	**	
Ref. days (D)	10	**	**	**	**	**	NS	NS	
R × D	10	**	**	**	**	NS	NS	NS	
R × S	2	*	**	**	**	**	NS	**	
D × S	20	**	**	**	**	NS	NS	NS	
R × S × D	20	**	NS	*	**	NS	NS	NS	
Error	132								

NS, Not significant, *$P<0.05$ and **$P<0.01$.

up to 4 days of refrigeration. The same was true in S_3 season over S_1 and S_2 se (table 4).

Reproductive rate was affected significantly ($P<0.01$) in all treatments, com to control (table 1). However, racewise comparison showed that newly ha larvae could be refrigerated in HM, for one day, without causing signi reduction in RR (table 5). In PM, there was no scope for refrigeration of larv it reduced RR significantly. Seasons x treatments interaction showed 2 days geration in S_3 season in PM and in all seasons in HM, was safe, as the differ in RR compared to control, were not significant. Refrigeration of newly ha larvae for 5 days resulted in 33·3% and 34·9% reduction in RR in PM and respectively. When refrigerated for 10 days, the corresponding reductions 71·7% in PM and 74·4% in HM. Since fecundity and male moth emergence not significantly different from the control and in respect of female moth emer and cocoon weight the effects were of lesser degree, the significant reduction in different treatments was mainly caused by poor survival and pupation rate

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vitro studies on uptake, storage and disappearance of norepinephrine spleen of white leghorn chicken

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Abstract. *In vitro* studies on uptake, storage and disappearance of norepinephrine were conducted on spleen slices of chicken. Uptake process was found to be time and concentration dependent. Maximum uptake of norepinephrine occurred at 10^{-4} M concentration after 45 min of incubation. Cocaine, metanephrine, phenoxybenzamine and a combination of cocaine and metanephrine, all inhibited the accumulation of norepinephrine, the last being most effective, indicating that both uptake$_1$ and uptake$_2$ processes are operative simultaneously and independently in disposition of amine. Disappearance studies following maximum accumulation indicated that the release was monophasic from a single pool. Reserpine pretreatment significantly reduced the accumulation and enhanced the release of norepinephrine.

Keywords. Norepinephrine; uptake; disappearance; chicken; spleen; cocaine; metanephrine; phenoxybenzamine; reserpine.

Introduction

ıe neurotransmitter of the sympathetic nerves in mammals is norepinephrine (NE) ıereas it is epinephrine (E) in amphibians. In poultry, the fact is yet to be tablished. Several investigators have reported higher levels of NE than E in avian ood and other tissues of chicks (Von Euler 1963; Ignarro and Shideman 1968; urkie *et al* 1970). However, others have reported higher levels of E than NE in rious tissues of chicken (Lin and Sturkie 1968; Kumar 1980; Uppal *et al* 1981; ına 1984). It has also been proposed that in this species a mixture of both NE and may be serving as neurotransmitter (De Santis *et al* 1975; Komori *et al* 1979). The echanisms involved in the synthesis, storage, uptake and release of adrenergic urotransmitter in various mammalian tissues have been studied extensively. But, poultry such information is not adequately available. The present study was, erefore, undertaken for gaining more insight in the uptake, storage and sappearance of NE in spleen slices of chicken.

Materials and methods

emale white leghorn chicken (20–25 weeks old) weighing between 1·5–2 kg were ed. The birds were acclimatized in the animal house of the department for 3–4 ıys before the start of experiment. Feed and water were provided *ad lib*.

The birds were sacrificed by decapitation, spleen was removed and kept in the e-cold Kreb's Hauseleit physiological salt saline (PSS). Slices of uniform thickness ıd diameter, weighing between 100–150 mg, were prepared with perspex tissue ınd microtome.

2.1 *Uptake of norepinephrine*

The spleen slices were preincubated for 15 min at 37°C in 10 ml of PSS containi iproniazid (10^{-4} M) and tropolone (10^{-4} M) as monoamineoxidase (MAO) a catechol-O-methyl transferase (COMT) inhibitors respectively. Thereafter, NE w added in 3 different concentrations (10^{-6} M, 10^{-5} M or 10^{-4} M) in different tul and incubated for varying intervals of 5, 10, 15, 30, 45 and 60 min. The slices treat with iproniazid and tropolone but not with NE were taken as controls. The N content in all the tissue slices was determined fluorometrically by the method Ansell and Beeson (1968) after extracting catecholamines in acid butanol described by Sadavongvivad (1970) using internal standard and the net uptake accumulated amine at various time intervals was calculated. Concentration curv were constructed by plotting the net uptake at different concentrations of ami against time. In the preliminary studies, the maximum accumulation of NE w observed at a concentration of 10^{-4} after 45 min of incubation. For all subseque studies, the spleen slices were incubated with 10^{-4} M concentration of NE 45 min.

2.2 *Disappearance of accumulated norepinephrine*

Following maximum accumulation, the slices were incubated in amine free mediu for different time intervals of 5, 10, 15, 30, 45 and 60 min. The NE content in the slices was measured as described earlier. Disappearance curves were constructed plotting the tissue amine concentration against time. Rate constant disappearance (K) and half-life ($t\frac{1}{2}$) of NE was calculated by regression analysis.

2.3 *Disappearance of accumulated NE from spleen slices in reserpine treated bird*

Birds were given reserpine at 5 mg/kg intramuscularly for two consecutive da Twenty four h after the second injection, the birds were sacrificed and *in vi* disappearance of accumulated NE in spleen slices was studied as described earlie

2.4 *Effect of drugs on uptake of NE*

After preincubation with iproniazid (10^{-4} M) and tropolone (10^{-4} M), the tiss was exposed to cocaine, metanephrine, phenoxybenzamine or a combination cocaine and metanephrine at a concentration of 10^{-4} M each. Thereafter, t uptake of NE was studied as described earlier.

3. Results and discussion

The accumulation of NE in spleen slices was found to be concentration and ti dependent, maximum being 8·62 μg/g ± 0·52 (SE) at 10^{-4} M concentration of N after 45 min of incubation (figure 1). The effect of various uptake blockers v cocaine, metanephrine, phenoxybenzamine and a combination of cocaine a metanephrine is shown in figure 2. Cocaine, a selective and competitive uptak blocker (Furchgott *et al* 1963; Farmer and Petch 1963), caused significant inhibiti

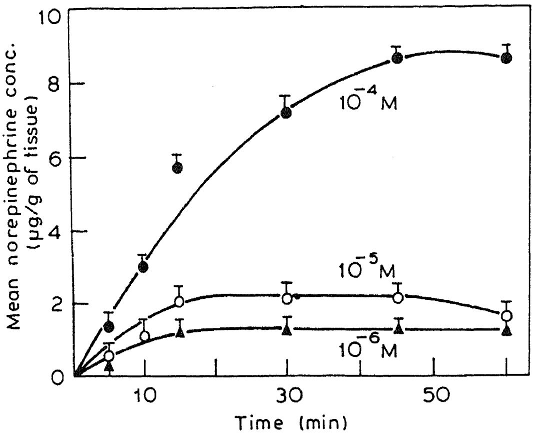

Figure 1. Accumulation of norepinephrine in spleen slices incubated with different concentrations of norepinephrine in the presence of tropolone and iproniazid (10^{-4} M each). Vertical bars denote $\pm$SE ($n=6$).

(59·83%) of NE uptake. Inhibition of uptake$_1$ by cocaine has been shown in chicken heart (Ignarro and Shideman 1968; Kumar 1980), liver (Agarwal *et al* 1987) and cat brain (Denglar *et al* 1961). Cocaine has also been reported to have no effect on uptake$_1$ process in rat uterus (Wurtman *et al* 1963) and chicken heart *in vivo* (Rana 1984).

Metanephrine, a potent uptake$_2$ blocker (Iversen 1965), exhibited 63·4% inhibition of accumulation of NE. Inhibition of uptake$_2$ process by metanephrine in other poultry tissues like heart (Kumar 1980; Rana 1984) and liver (Agarwal *et al* 1987) has also been reported.

The above results indicated that both uptake$_1$ and uptake$_2$ processes are almost equally involved in terminating the effects of exogeneous NE. The maximum inhibition of 79·19% was observed with the combination of cocaine and metanephrine further indicating that both uptake$_1$ and uptake$_2$ processes are operating simultaneously. Further, phenoxybenzamine which is known to inhibit both uptake$_1$ and uptake$_2$ processes nonspecifically (Axelrod *et al* 1962; Lightman and Iversen 1969) exhibited 66·67% inhibition of NE accumulation supporting the above results. Similar observations on chicken heart (Kumar 1980; Rana 1984) and liver (Agarwal *et al* 1987) are on record. The fact that the combination of cocaine and metanephrine or phenoxybenzamine could not cause complete inhibition of accumulation of NE indicates that some other process may also be involved in dissipating NE in chicken spleen.

In the spleen slices obtained from reserpine treated birds, a significantly lower accumulation (5·20 µg/g$\pm$0·37 SE) of NE was found as shown in figure 3. However, the disappearance of accumulated NE was linear with respect to time in normal as well as in reserpine treated birds (figure 3). The elimination was faster in spleen slices from reserpinised birds as compared to the normal birds. The disappearance rate constants were found to be $2{\cdot}80\times10^{-2}$ min^{-1} and $3{\cdot}50\times10^{-2}$ min^{-1} and the elimination half lives were 24·57 and 19·80 min in normal and reserpine treated birds, respectively. The significant reduction of catecholamines accumulation in reserpine pretreated chicken heart (Kumar 1980; Rana 1984) and liver (Agarwal *et al*

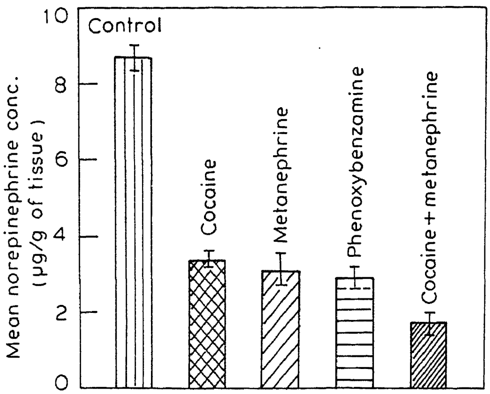

Figure 2. Accumulation of norepinephrine in spleen slices of 45 min incubation in the absence and presence of various uptake blockers (10^{-4} M). Vertical bars denote ±SE ($n=6$).

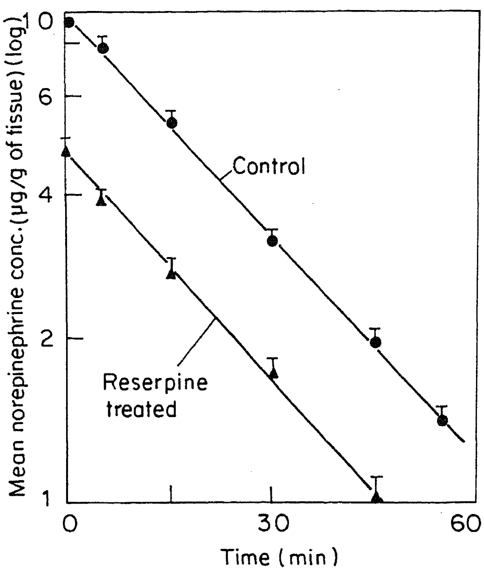

Figure 3. Disappearance of norepinephrine in control and reserpine treated chicken slices after maximum accumulation of norepinephrine. Vertical bars denote ±SE ($n=6$).

1987) has also been reported from our laboratory. Rana (1984) in the *in vivo* studies in chicken heart, reported a biphasic release of recemic NE. In a number of mammalian tissues, Montanari *et al* (1963) also reported similar type of biphasic release. Contrary to these, a monophasic disappearance linear with respect to time was observed in the present investigation. Similar monophasic linear release of NE has been reported in chicken heart (Kumar 1980) and liver (Agarwal *et al* 1987).

The present study is an attempt to fill the gap in our knowledge of how NE is handled by the sympathetic nerves in the chicken spleen. The results show some interesting variations in this species as compared to mammals. The differences

tween spleen and other poultry tissues like heart and liver, are not very gnificant.

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ırosecretion of brain and thoracic ganglion and its relation to roduction in the female crab, *Potamon koolooense* (Rathbun)

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Abstract. The gonadotropic function of the neurosecretory system of brain and thoracic ganglion in the adult female crab, *Potamon koolooense* is examined. The type A-cells of brain and thoracic ganglion display marked annual cyclic changes of synthesis and release of cytoplasmic material in association with the ovarian cycle. Administration of the thoracic ganglion extract during early oogenesis induced oocyte growth and precocious vitellogenesis, with an increase in the ovarian weight or gonad index and oocyte diameter. However, the brain extract injection did not produce marked changes in the immature ovary. The results suggest that the ovarian activity, particularly growth and vitellogenesis, depend on the neurosecretion of the thoracic ganglion, especially on the activity of A-cells. The neurosecretion of A-cells of the brain seems to be involved in the process of mating and ovulation.

Keywords. Neurosecretion; ovary; brain; thoracic ganglion; crab.

Introduction

ːral investigations have been made to find out neuroendocrine actions involved the control of ovarian maturation in crustaceans. As a result, evidence cerning the source of ovary-inhibiting hormone (the eyestalks) and its specific cts have been produced (Adiyodi and Adiyodi 1970; Charniaux-Cotton 1985). vever, relatively little is known about the source and mechanism of other rohormones, such as the ovary-stimulating hormone of the decapods. ːological (Matsumoto 1958; Perryman 1969; Deecaraman and Subramonium 3; Mirajkar *et al* 1983) and experimental (Gomez 1965; Blanchet-Tournier 1982) ences indicate that brain elaborates an ovary-stimulating hormone. On the ːr hand similar studies on some decapods have provided evidence that an ovary-ıulating hormone is also released by the thoracic ganglion (Parmeswaran 1956; tsumoto 1958; Otsu 1963; Gomez 1965; Hinsch and Bennet 1979; Rao *et al* 1981; caraman and Subramonium 1983; Dehn *et al* 1983; Sarojini and Gyananath 5).

ecent studies have indicated that the ovary stimulating hormone of brain or ːacic ganglion stimulates formation and utilization of yolk protein in the oocytes usy 1980; Dehn *et al* 1983). The purpose of this study is to examine the adotropic function of the brain and thoracic ganglion in female *Potamon looense*. The ovarian cycle and the cytology of the neurosecretory cells of brain thoracic ganglion of *P. koolooense* have been studied earlier (Joshi and Khanna 2, 1984, 1985).

2. Materials and methods

Adult female *P. koolooense* (carapace width, 40–45 mm) were collected every month throughout the year from a stream at Pithoragarh. The brain and thoracic ganglion were fixed in Bouin's fluid. Serial sections in different planes were cut at 6 μm with paraffin method and stained with aldehyde fuchsin (AF), Bargmann's chrom-alum haematoxylin phloxin (CHP) (Pearse 1977), alcian blue phloxin (ABP) and Azan. To estimate the secretory activity of the neurosecretory cells, the cell and nuclear sizes and the degree of cytoplasmic granulation were estimated. The cells are ellipsoid and those having complete nuclei were selected for the estimation of cellular and nuclear diameters (average of short and long axis) with ocular micrometer. Measurement of diameters was carried out on 4 sections from each of 10 crabs in each month.

For experiments, adult female crabs were collected in July. Forty five crabs of intermoult stage (carapace width, 40–45 mm) were selected and divided into 3 groups of 15 animals each. Each specimen of first and second group was injected with 0·15 ml of brain and thoracic ganglion extract respectively at every 3 day interval. The crabs of third group were injected with distilled water which served as concurrent controls. Aqueous extract of brain was prepared by grinding the contents of freshly removed 10–12 brains suspended in 3 ml of distilled water and centrifuging for 5 min. The supernatant was used for injections. The thoracic ganglion extract was similarly prepared and injected. The extracts were injected through the arthropodial membrane at the base of 4th leg. The crabs of different groups were maintained in identical conditions of room temperature and daylength in separate aquaria, containing freshwater 4 cm deep. The crabs were fed on earthworms, and water was changed every 3 to 4 days. No moulting was observed in the control and treated crabs.

Before treating the crabs with brain or thoracic ganglion extract, 5 specimens were taken for examination of ovarian condition and these served as the initial controls or normals. Thereafter, crabs of first and second group were sacrificed at 10th (after 3 injections), 21st (after 6 injections) and 30th day (after 8 injections) of the experiment. Five crabs from each group were sacrificed at each autopsy. An equal number of crabs of third group (concurrent controls) were also sacrificed at 10th, 21st and 30th day of the experiment. The body weights and ovarian weights of sacrificed crabs were recorded and gonosomatic index (GSI) was calculated using the formula:

$$\mathrm{GSI} = \frac{\text{Ovarian weight}}{\text{Body weight}} \times 100.$$

For histological and histometric observations, pieces of ovaries were fixed in Bouin's fluid and stained with Delafield's haematoxylin-eosin. For general identification of yolk bodies, alcian blue-naphthol yellow and congo red technique were adopted for glycoproteins and Sudan black B for lipids (Pearse 1977). Oocytes having complete nuclei and appearing rounded in sections were selected for the estimation of oocyte diameter with ocular micrometer. The measurement of diameters was carried out on 4 randomly selected histological sections from each crab. The results are expressed as mean $\pm$ SD from 5 specimens in each group (table 2).

3. Results

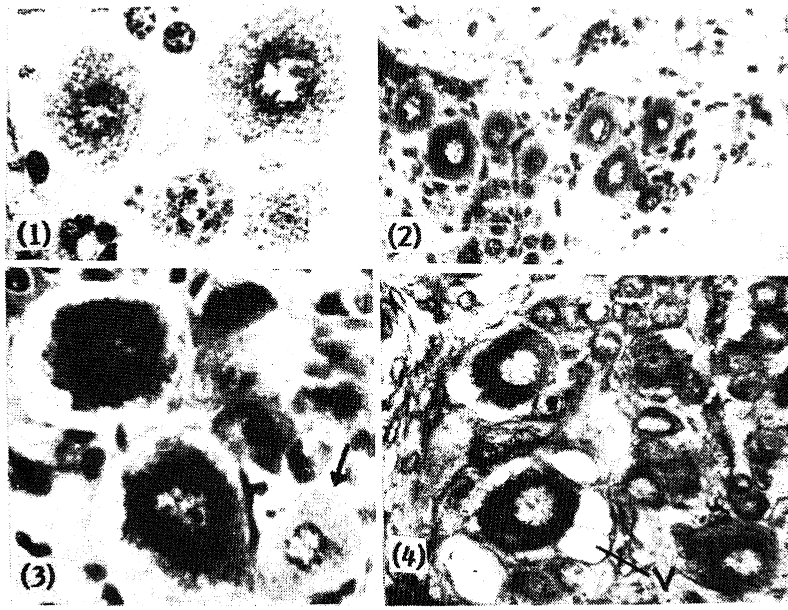
The histological study has shown the presence of 4 types of neurosecretory cells (A, B, C and D cells) in the brain and thoracic ganglion of *P. koolooense* (Joshi and Khanna 1984, 1985). The present observations (table 1) indicate that only the type A-cells in the brain and thoracic ganglion exhibit striking seasonal cyclic changes in correlation with the ovarian cycle. The B, C and D cells were not found to show definite annual secretory cycle that can be correlated with the ovarian cycle; the cellular size and the density of cytoplasmic material of these cells varies from time to time, and sometimes in the individuals of the same month. The increase of cell and nuclear diameters and the production of cytoplasmic granules in the A-cells of brain starts in December and progresses through January to March (table 1, figures 2, 3), when oocytes attain maturity. The hypertrophied cells with large number of granules and some vacuoles in the cytoplasm (active state of secretion) are observed in March–April (prebreeding phase), remaining almost the same till mating and ovulation (May or June) (figure 4). After that the activity of the cells decreases substantially, and very little neurosecretion remains in the cytoplasm (figure 1).

In the thoracic ganglion, the increase in cell and nuclear diameter and accumulation of stainable neurosecretion within the A-cells starts during ovulation period (May to July) (table 1, figure 6). Cells with abundant secretory granules and few vacuoles are observed during July–August, when oogenesis starts in the ovaries. During this period, ovaries contain several oogonia (mitotic multiplication), previtellogenic oocytes and a few primary vitellogenic oocytes. Extensive vacuolation of the cytoplasm was observed during September to December or January (figures 7, 8), when ovaries contain a large number of secondary and tertiary vitellogenic oocytes (active protein vitellogenesis). After that the cells appear shrunken and almost devoid of stainable granules (figures 5, 8). This shows that the cell activity is depressed with termination of vitellogenesis (or ripening of oocytes).

Table 1. Seasonal cytometric and neurosecretory material (NSM) intensity changes in the A-cells in brain and thoracic ganglion of *P. koolooense*.

	Brain			Thoracic ganglion		
Month	Cell size (μm)	Nuclear size (μm)	NSM intensity	Cell size (μm)	Nuclear size (μm)	NSM intensity
Jan	66·15 ± 2·9	22·60 ± 1·38	+ +	105·40 ± 3·50	19·32 ± 1·26	+
Feb	69·70 ± 3·2	25·31 ± 1·41	+ +	87·58 ± 3·15	17·05 ± 1·18	+
Mar	70·42 ± 3·1	26·75 ± 1·37	+ + +	80·42 ± 3·05	17·38 ± 1·22	+
Apr	81·25 ± 3·3	28·40 ± 1·38	+ + + +	72·35 + 3·15	15·17 ± 1·05	+
May	90·76 ± 3·6	30·25 ± 1·40	+ + + +	86·75 ± 3·50	20·00 ± 1·15	+ + +
June	78·10 ± 2·8	25·20 ± 1·36	+ + +	102·15 ± 3·40	23·85 ± 1·13	+ + +
July	61·18 ± 2·9	20·52 ± 1·32	+	125·76 ± 3·82	25·45 ± 1·15	+ + + +
Aug	54·82 ± 2·8	16·00 ± 1·27	+	136·85 ± 4·30	26·76 ± 1·36	+ + + +
Sept	52·33 ± 2·3	15·50 ± 1·26	+	143·35 ± 4·62	29·75 ± 1·60	+ + +
Oct	53·45 ± 2·6	16·00 ± 1·21	+	151·82 ± 5·80	28·28 ± 1·35	+ + +
Nov	52·65 ± 2·5	16·80 ± 1·27	+	140·36 ± 4·85	28·50 ± 1·05	+ +
Dec	60·10 ± 2·7	18·65 ± 1·35	+ +	130·27 ± 4·25	26·15 ± 1·25	+ +

+ = Less; + +, + + + = moderate; + + + + = high.



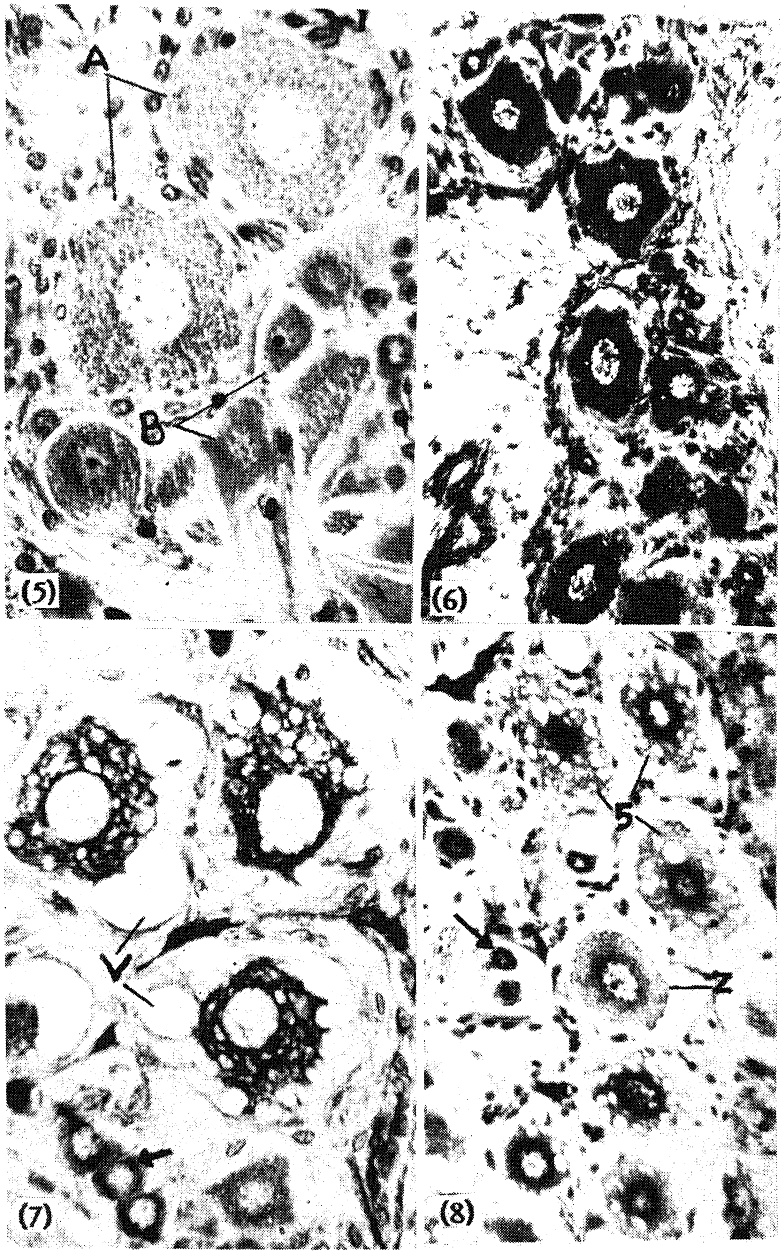
Figures 1–4. Portions of sections of brain. **1.** Very few granules in A-cel (CHP × 400). **2.** Accumulation of granules in A-cells in January. The small typ also seen (CHP × 140). **3.** Abundance of cytoplasmic material in A-cells in M are poorly stainable due to scarcity of cytoplasmic granules (arrow) (A Abundance of granules and vacuolation (V) of A-cell cytoplasm in May. B-c seen (AF × 400).

3.1 *Effect of brain and thoracic ganglion extract on ovaries*

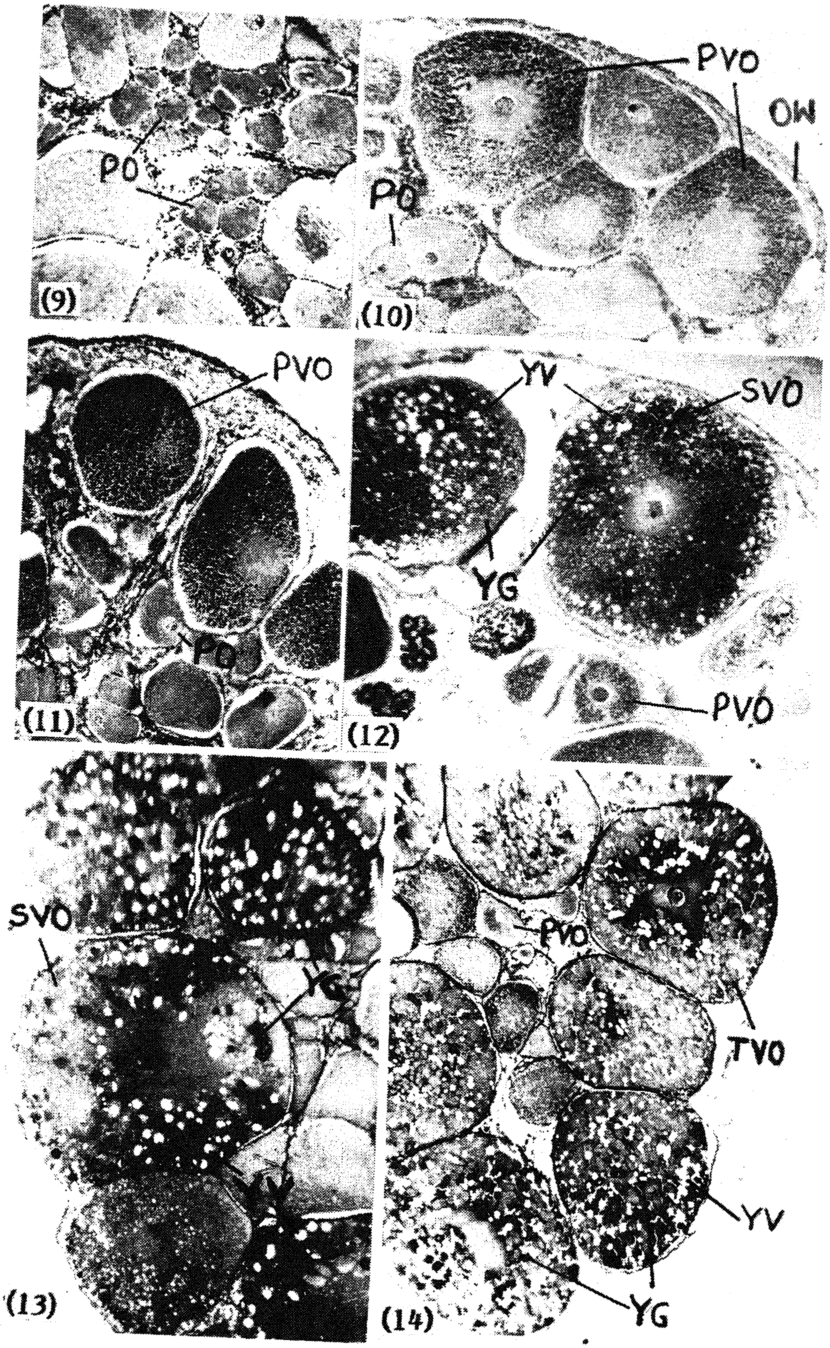
3.1a *Ovaries of normal crabs*: At the initiation of experiment the ovari early phases of recrudescence as indicated by the preponderance of oo vitellogenic oocytes (figure 9). In the concurrent controls, the oogenesis minimal at all periods studied (10th, 21st and 30th day), as demons histological (figure 10) and morphometric observations (table 2). Th appeared white or pale yellow, and contained oogonia, previtellogenic oc few primary vitellogenic oocytes.

3.1b *Ovaries of brain extract injected crabs*: The treatment produced n effect on the ovaries. The histology of treated crabs (figure 11) sacrifice 21st and 30th day of experiment was similar to that observed in the Furthermore, the ovarian weight, gonad index and oocyte diameter of t and treated crabs were similar (table 2).

3.1c *Ovaries of thoracic ganglion extract treated crabs*: Injections o ganglion extract induced an increase in ovarian weight, gonad index a diameter within 10 days of treatment (table 2). The values further increase sacrificed at 21st and 30th day of the experiment (table 2). The colour of t changed progressively from deep yellow (10th day) to orange (3



Figures 5–8. Portions of sections of thoracic ganglion. **5.** Very few granules in A-cells and granulated B-cells in March (CHP × 380). **6.** Abundance of granules in A-cells in June (CHP × 140). **7.** Vacuoles (V) in the cytoplasm of A-cells in September. The granulated B-cells are also seen (arrow) (AF × 380). **8.** Degranulated (Z) and vacuolated A-cells in December. B-cells are also seen (CHP × 140).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Figures 9–14.

Table 2. Effect of brain and thoracic ganglion extract injection on the ovaries during post-spawning period (July 10–August 9, 1980).

Group	Duration (days)	Body weight (g)	Ovarian weight (g)	Gonad index	Oocyte diameter (mm)
Initial control		28·15 ± 1·60	0·19 ± 0·02	0·79 ± 0·03	0·24 ± 0·02
Concurrent	10	28·00 ± 1·5	0·20 ± 0·02	0·80 ± 0·03	0·22 ± 0·01
Control	21	26·46 ± 1·24	0·19 ± 0·01	0·81 ± 0·04	0·24 ± 0·02
	30	26·82 ± 1·30	0·16 ± 0·02	0·75 ± 0·03	0·25 ± 0·02
Brain	10	26·65 ± 1·26	0·17 ± 0·01	0·79 ± 0·04	0·22 ± 0·02
extract	21	28·35 ± 1·50	0·18 ± 0·02	0·81 ± 0·05	0·23 ± 0·02
injected	30	27·75 ± 1·40	0·21 ± 0·02	0·82 ± 0·04	0·25 ± 0·03
Thoracic ganglion	10	27·66 ± 1·27	0·35 ± 0·03	1·86 ± 0·31	0·51 ± 0·06
extract	21	28·48 ± 1·32	0·52 ± 0·04	2·88 ± 0·36	0·66 ± 0·05
injected	30	27·18 ± 1·48	0·71 ± 0·03	2·82 ± 0·48	0·76 ± 0·08

Histologically, the ovaries at 10th day of experiment showed oogonial proliferation and premeiotic oocytes in the central germinal area and growth of several previtellogenic oocytes into primary (lipid vitellogenesis) and secondary vitellogenic oocytes (lipid and protein vitellogenic) in the peripheral region of the ovary (figure 12). At 21st day of experiment, the ovaries contained oocytes in the phase of lipid and protein vitellogenesis (secondary vitellogenic oocytes) (figure 13). Their ooplasm consisted of a large number of unstainable yolk vacuoles or vesicles and eosinophilic yolk globules. At 30th day of experiment, the ovaries contained a large number of oocytes in the phase of protein vitellogenesis (tertiary vitellogenic oocytes). This is the late phase of vitellogenesis, and oocytes become filled almost completely with acidophilic yolk globules and unstainable yolk vesicles (figure 14).

Thus, at early oogenesis (when ovaries contained several oogonia and previtellogenic oocytes and few primary vitellogenic oocytes), thoracic ganglion extract induce growth of oogonia into oocytes and accelerates formation of yolk in growing oocytes (precocious lipid and protein vitellogenesis). The progressive changes in the colour and weight of the ovaries and oocyte size following thoracic ganglion extract treatment may be due to the accumulation of yolk.

Figures 9–14. Portions of sections of ovaries. Haematoxylin-eosin (× 40). **9.** Occurrence of previtellogenic oocytes (PO) in the ovaries in initial control crab. **10.** Ovary of concurrent control at 30th day. Note the occurrence of previtellogenic oocytes (PO) in the central germinal zone and primary vitellogenic oocytes (PVO) towards peripheral area of the ovary. **11.** Ovary of brain extract treated crab at 30th day. Note the occurrence of previtellogenic (PO) and primary vitellogenic oocytes (PVO). **12.** Ovary of thoracic ganglion extract treated crab at 10th day. Note the occurrence of primary vitellogenic oocytes (PVO) and secondary vitellogenic oocytes (SVO). **13.** Ovary of the thoracic ganglion extract treated crab at 21st day. Note the presence of secondary vitellogenic oocytes (SVO). **14.** Ovary of the thoracic ganglion extract treated crab at 30th day. Note the occurrence of primary vitellogenic oocytes (PVO) in the central germinal area and tertiary vitellogenic oocytes (TVO) towards peripheral region of the ovary. OW, Ovarian wall; YV, yolk vesicles; YG, yolk globules.

4. Discussion

Experiments involving implantation of cerebral (Gomez 1965) or thoracic ganglion (Otsu 1963; Gomez 1965; Hinsch and Bennet 1979) suggest the existence of a gonad-stimulating hormone in these ganglia. The results of present investigation indicate that the neurosecretion of brain and thoracic ganglion serve a similar function in female *P. koolooense*. The variations in the activity of A-cells of the brain and thoracic ganglion are related to the ovarian activity. During periods of ovarian maturation the brain A-cells become hypertrophied and the neurosecretory material accumulates within their perikarya. Discharge of the secretory material occurs just prior to and during ovulation. In the brain of other decapods studied so far, the A-cells (Matsumoto 1958; Mirajkar *et al* 1983), type 3 cells (Perryman 1969) and type C-cells (Deecaraman and Subramonium 1983) appear maximally active during ovarian maturation, as well as during ovulation. As shown in table 1, variation in the activity of type A-cells of thoracic ganglion is related to the oocyte development. That is, the cells start releasing their secretory material when oocytes proceed from previtellogenesis to vitellogenesis. The activity reaches a maximum during yolk formation, especially during protein vitellogenesis. As the vitellogenesis terminates, the cell activity slows down substantially.

The A-cells of thoracic ganglion pour their secretion much earlier than the A-cells of brain; the A-cells of thoracic ganglion release their material during yolk formation, whereas the brain A-cells appear maximally active during final phases of ovarian cycle (ovulation). Consequently, it is possible that the thoracic ganglion A-cells stimulate oocyte growth and yolk deposition, whereas the brain A-cells seem to initiate mating and ovulation or oviposition. A higher activity (vacuolation of cytoplasm) during ovarian maturation has also been observed in A-cells in the thoracic ganglion of *Paratelphusa hydrodromous* (Parmeswaran 1956; *Potamon dehaani* and *Neptunus trituberculatus* (Matsumoto 1958); *Macrobrachium lanchesteri* (Rao *et al* 1981); *Macrobrachium kistensis* (Mirajkar *et al* 1983) and B-cells in the thoracic ganglion of *Squilla holoschista* (Deecaraman and Subramonium 1983).

The administration of thoracic ganglion extract during early phases of oogenesis precipitated oocyte growth and precocious vitellogenesis, apparently increasing the ovarian weight and oocyte diameter. After treatment, the ovarian colour changed progressively from white through yellow to orange, which is associated with the vitellogenesis. Similar maturational changes have also been observed in the ovaries of other crustaceans after treatment with thoracic ganglion extract (Deecaraman and Subramonium 1983; Sarojini and Gyananath 1985) or with thoracic ganglion implantation (Otsu 1963; Gomez 1965; Hinsch and Bennet 1979). However, in the present study, injection of brain extract did not produce perceptible modification in the ovaries of treated crabs, when compared to that of the controls. The extract was prepared from brains of crabs collected in July and injected into the intact crabs during this period. Since A-cells of brain normally contain very few stainable granules during July–August, a low concentration of the hormonal factor in the extract may possibly be the reason for the absence of obvious changes in the ovaries after the brain extract treatment.

The present results suggest that the ovarian activity, particularly the oocyte growth and vitellogenesis, depend on the neurosecretion of thoracic ganglion, especially on the activity of A-cells. Furthermore, present histological observations

ndicate that the neurosecretion of brain A-cells may function in the final phases of emale reproductive cycle (mating and ovulation). However, further studies on this species are desirable to see whether or not neurosecretory material acts directly on he ovaries.

Acknowledgements

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Sublethal effects of organochlorine insecticide (endosulfan) on protein, carbohydrate and lipid contents in liver tissues of *Oreochromis mossambicus*

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Abstract. Endosulfan, a chlorinated hydrocarbon insecticide was tested against the fish *Oreochromis mossambicus*. The median lethal concentrations for 24, 48, 72 and 96 h were 0·02, 0·012, 0·007 and 0·006 ppm respectively. The fish were exposed to sublethal concentrations for about 10 days and the protein, carbohydrate and lipid contents in the liver tissue during this period was estimated. There is a general decline in the level of protein, carbohydrate and lipid which is due to the increasing length of exposure to endosulfan.

Keywords. Sublethal effects; endosulfan; liver tissues; *Oreochromis mossambicus*.

1. Introduction

Pesticides have been one of the most effective weapons discovered by man to protect agricultural products from the attack of pests. But the extensive use of pesticides pose a constant threat to the aquatic life by altering the habitat, behaviour pattern, growth and reproductive potential (Jarvinen *et al* 1977; Anderson and Peterson 1969). Although there are considerable research activities in the field of pesticides, there is wide variation in the amount of information available concerning the effect of particular pesticides on selected non-target organisms. Among the organisms studied, fishes have drawn more attention due to their economic importance. *Oreochromis mossambicus*, an economically important fish, abundantly available in local water bodies and is a valuable protein-rich source of food for humans. Lack of information on the toxicity and sublethal effects of endosulfan on the biochemical composition of the species mentioned has induced the authors to conduct the present study.

2. Materials and methods

Oreochromis mossambicus weighing approximately 10 g were collected from ponds in and around Madurai Kamaraj University. They were maintained in large cement tanks at 30 ± 2°C for atleast two weeks before use, during which they were regularly fed with minced goat liver. The water used was clear, unchlorinated having 5·6–8·4 ppm of dissolved oxygen and pH range from 7·6–7·8. During the period of experiment the fishes were not fed.

Lethal toxicity of endosulfan (thiodan, 94% active ingradient) was calculated by conducting static method (APHA 1971). The pesticide formulation in acetone was serially diluted with water to obtain the desired concentrations of 0·005, 0·007, 0·009, 0·01, 0·02, 0·04, 0·05 and 0·1 ppm endosulfan. Healthy fish were chosen at

random from the acclimation tanks and 10 numbers were introduced into 15 L experimental glass troughs. A control was kept without endosulfan. Mortality was recorded at every 30 min intervals up to 24 h, 3 h intervals up to 48 h and 6 h intervals up to 96 h. LC_{50} values were calculated for the stipulated time (24, 48, 72 and 96 h) by plotting percentage mortality against the concentration of the pesticide. As there was no mortality in the control there was no need for any correction.

For biochemical studies, *O. mossambicus* were exposed to sub-lethal concentrations of 0·003, 0·004 and 0·005 ppm endosulfan. Ten animals were kept at each concentration for the period of 4, 7 and 10 days. Atleast two replicate troughs were maintained for each concentration. Control fish were reared in pesticide-free water. All the fish were regularly fed with minced goat liver during the experimental period. After exposure, fish from each group were sacrificed and the liver were pooled. Carbohydrate, protein and lipid contents of the liver were then estimated by the methods of Dubois *et al* (1956), Lowry *et al* (1951) and Bligh and Dyer (1959) respectively.

3. Results and Discussion

The median lethal concentrations calculated for 24, 48, 72 and 96 h were 0·02, 0·012, 0·007 and 0·006 ppm respectively (figure 1). Fish survived at 0·005 ppm and below, indicating that 0·005 ppm is the sub-lethal level. The 96 h LC_{50} values of endosulfan noted for *Barbus stigma* was 0·0043 ppm (Manoharan and Subbiah 1982). Using endosulfan, Amminikutty and Rege (1977) observed that 0·016 ppm is the LC_{50} value for the fish *Gymnocorymbus ternetzi.* Singh and Narain (1982) noticed variations in 96 h LC_{50} on the cat fish *Heteropneustes fossilis* in relation to season, size and weight of the fish. Rao and Murty (1982) demonstrated in 3 species of cat fish that the relative toxicity between species could not be determined using LC_{50} values alone, the slopes of endosulfan toxicity curves were different for different species. The data obtained in the present work indicates that different fishes have different tolerance range against the toxic effects of the same pesticide. In this way *O. mossambicus* seems to be more tolerant to endosulfan than *B. stigma.* The higher tolerance of this fish to this pesticide is basically due to species and size variations.

Fish constitutes one of the major sources of cheap nutrition for the human beings (Bhagavathi and Rath 1982). The nutritional value of different fishes depends on their biochemical compositions like protein, amino acids, vitamins, mineral contents etc. yet biochemical changes induced in the different tissues of fish by pollutant have not been studied in significant details (Natarajan 1981; Murty and Devi 1982). In the present investigation, liver protein content of the fish exposed to 0·003, 0·004 and 0·005 ppm of endosulfan showed a general increase over the control on the 4th day. The magnitude of increase was directly proportional to the concentration of the pesticide. Then it started declining from the initial level towards the control level on the 7th day. Liver protein content of the treated fish on the 10th day showed a steady decline below the control level. Here the decrease in the protein content was directly proportional to the length of exposure (table 1). This result stands in good agreement with the observation reported by Manoharan and Subbiah (1982). Similar observations were also noted when fish were exposed to pollutants (Rath and Misra 1980). The decline in protein suggests an intensive

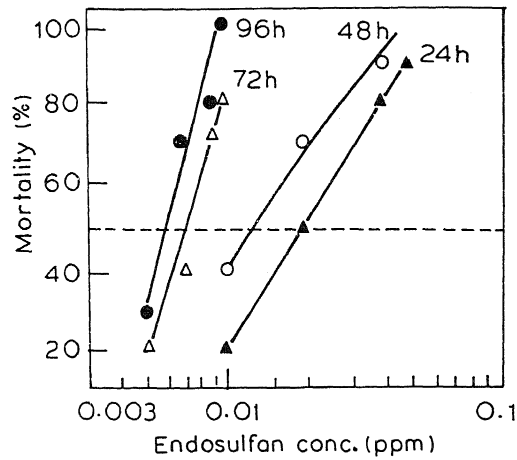

Figure 1. LC_{50} of *O. mossambicus* exposed to endosulfan for different durations at 30 ± 2°C.

Table 1. Effect of sub-lethal concentrations of endosulfan on liver protein content of *O. mossambicus*.

Duration of exposure (days)	Pesticide concentration (ppm)	Liver protein content (mg/g liver dry wt)
4	Pesticide free water	9·9 ± 0·1
	0·003	11·2 ± 0·09
	0·004	13·2 ± 0·08
	0·005	14·6 ± 0·1
7	Pesticide free water	10·6 ± 0·12
	0·003	10·8 ± 0·1
	0·004	12·4 ± 0·007
	0·005	14·4 ± 0·09
10	Pesticide free water	11·4 ± 0·1
	0·003	4·7 ± 0·08
	0·004	5·2 ± 0·09
	0·005	8·2 ± 0·1

proteolysis which in turn could contribute to the increase of free amino acids to be fed into the tricarboxylic acid cycle as keto acids, thus supporting the hypothesis of Kabeer (1979).

The lipid content of liver decreased in the pesticide treated fish as in the case of protein (table 2). Decrease in the lipid content in the present study might be due to the utilization of lipids for the energy demand associated with the situation of stress (Rao and Rao 1981; Harpert *et al* 1977).

There was a general decline in the liver carbohydrate content of fish exposed to sublethal concentrations of endosulfan. Here the carbohydrate content dropped significantly below the control level with an increase in the pesticide concentration after 4, 7 and 10 days of exposure (table 3). This confirms the findings of Murthy and Devi (1982). Similar trend was also observed in endosulfan treated *Barbus*

Table 2. Effect of sub-lethal concentrations of endosulfan on liver lipid content of *O. mossambicus.*

Duration of exposure (days)	Pesticide concentration (ppm)	Liver lipid content (mg/g liver wet wt)
4	Pesticide free water	30·0 ± 0·18
	0·003	35·0 ± 0·37
	0·004	40·0 ± 0·21
	0·005	50·0 ± 0·31
7	Pesticide free water	33·0 ± 0·32
	0·003	30·0 ± 0·41
	0·004	32·0 ± 0·28
	0·005	34·0 ± 0·19
10	Pesticide free water	36·0 ± 0·26
	0·003	20·2 ± 0·21
	0·004	29·5 ± 0·34
	0·005	32·5 ± 0·29

Table 3. Effect of sub-lethal concentrations of endosulfan on liver carbohydrate content *O. mossambicus.*

Duration of exposure (days)	Pesticide concentration (ppm)	Liver carbohydrate control (mg/g liver dry wt)
4	Pesticide free water	22·0 ± 0·21
	0·003	23·0 ± 0·18
	0·004	24·0 ± 0·19
	0·005	24·0 ± 0·2
7	Pesticide free water	45·0 ± 0·26
	0·003	42·0 ± 0·24
	0·004	41·0 ± 0·21
	0·005	37·0 ± 0·19
10	Pesticide free water	50·0 ± 0·3
	0·003	39·0 ± 0·28
	0·004	36·0 ± 0·19
	0·005	27·0 ± 0·2

stigma (Manoharan and Subbiah 1982). Umminger (1970) found that carbohydrates represent the principal and immediate energy precursors for fishes exposed to stress conditions while proteins being the energy source to spare during chronic periods of stress.

Generally more energy is needed to mitigate any stress condition. This energy may be obtained from carbohydrates, proteins and/or lipids.

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Factors affecting ant (Formicidae: Hymenoptera) visits to the extrafloral nectaries of *Croton bonplandianum* Baill

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Abstract. *Croton bonplandianum* invites a set of ant species to its extrafloral nectaries. The composition and frequency of these nectariferous ants on the plant were distinctly different from those on the ground. We have investigated the factors that govern visitation pattern of the ant species to the plant.

The available pattern of nectar, and the age-related increase in the nectar content influence the ant visitation. Availability of nectar in small dispersed units specifically encourages the visits of individual foragers and discourages the group foragers. Increase in the nectar content of the plant with aging, favours the host occupation by the nomadic ants, *Tapinoma melanocephalum* F. A few species such as *Pheidole woodmasoni* Forel could not harvest the nectar in the plant due to their inability to climb. Differences in the habitat preferences of ants affect their composition on the host plants.

Keywords. *Croton bonplandianum*; ant-plant mutualism; seed dispersal; nectar presentation.

1. Introduction

Flowering plants display mutualistic relations with ants for various advantages like protection against pests (Janzen 1966; Bentley 1977a; Schemske 1980) and dispersal of seeds (O'Dowd and Hay 1980; Ganeshaiah and Umashanker 1988). For this purpose, plants attract ants by offering them shelter, protein bodies (Janzen 1966) and nectar (Bentley 1976, 1977a, b). However, the attraction of ants by plants may not be restricted to a few species (Schemske 1982); for instance, all the nectariferous ants may visit extrafloral nectaries. Such random or undirected visits may not be profitable to the plant, especially if the ant species differ in their ability to reciprocate rewards. Hence selection might favour the development of special plant features that regulate the ant species visiting the plant in order to maximise their benefits; besides, certain specific behavioural traits of ants may also contribute to such a specificity.

In this paper, we investigate these aspects in a mutualistic system between *Croton bonplandianum* Baill (hereafter Croton) and a set of nectariferous ants, where a weak mutualism exists for the dispersal of seeds (Ganeshaiah and Umashanker 1988). More specifically, we attempt to answer two questions:

(i) In a weak mutualistic association like the Croton—ant system, is there any specificity in the ant visits to the plant?
(ii) What features of the host and the ants contribute to such a non-random ant visits? We, however, have not attempted to test the adaptive significance of the plant to such specificity in ant visitation.

2. System

Croton is a monoecious annual herb and bears extrafloral nectaries on the stalks of developing fruits. The plant produces fruits in different successions called stages, each stage lasting for about 20 days (Umashanker and Ganeshaiah 1984). The number of nectaries per plant increases with each stage because of increase in both number of fruits per inflorescence and number of inflorescences per plant (table 1). The growth of the plant almost ceases after IV or V stage by when the plant bears about 110 inflorescences. Since the total number of nectaries in each plant would be constant after IV stage, only IV and V stage plants were used except when specified. Croton attracts a set of nectariferous ants which aid in seed dispersal (Ganeshaiah and Umashanker 1988).

3. Materials and methods

The study was conducted in a 10 acre plot along the ravines adjoining the UAS campus (Hebbal, Bangalore). Croton has established itself here in huge colonies atleast for the past 7–8 years (Umashanker and Ganeshaiah 1984). Based on the distribution of plants, the study area was divided into dense (5–10 plants/m^2) and sparse (less than 1 plant/m^2) zones. A preliminary census in the study area indicated that ant activity was high between 0800 and 1000 h, and 1600 and 1800 h. Hence observations were recorded during these hours of the day for 3 months avoiding cloudy and rainy days.

3.1 *Ant census*

Five plants of IV and V stages were randomly selected in each zone during each visit to experiment-plot; the species and the number of ants on each of these plants were recorded. The census of nectariferous ants was done by counting the number of ants attracted to cotton swabs soaked with 0·5 ml sugar solution (20% sucrose + 1% glycerine) displayed randomly on the ground 2–3 m away from the croton plants for 20–30 min. Another set of 30 plants of all the 5 stages were randomly selected and their ant composition and nests, if any, under these plants were also recorded.

3.2 *Effect of splitting the nectar quantity*

The nectaries in Croton are sparsely distributed. To test if this split pattern of nectar availability in the plant differentially attracts group and individual foragers, cotton swabs soaked with 0·5 ml of sucrose solution were displayed either entirely as a single unit or in 10 split units at a distance of 1 m around the nests of two ant species, viz. *Monomorium indicum* (individual forager) and *Pheidole woodmasoni* (group forager). Five nests of each species were chosen and each was provided with one of the two patterns of nectar availability at a time, in a random sequence. The experiment was repeated for 10 days assigning the two treatments randomly to the nests everyday. The number of ants visiting each unit of cotton swab in 15 min was recorded.

Table 1. Age, height, number of inflorescences and nectaries per inflorescence of different stages of *C. bonplandianum*.

Stage	Age (days)	Plant height (cm)	Inflorescence		Nectaries per inflorescence
			Mean	Range	
I	20–25	16.4 ± 0.8	1·00	—	2.3 ± 0.06
II	35–4040	25.5 ± 0.8	3·52	3–6	4.0 ± 0.11
III	50–60	33.7 ± 0.2	15·32	10–22	6.4 ± 0.18
IV	65–75	38.6 ± 0.6	52·20	40–85	7.5 ± 0.36
V	75 and above	44.3 ± 2.9	113·00	90–260	8.5 ± 0.32

3.3 *Effect of elevation of nectar*

Nectar in the plant is available at a height of 16–45 cm above the ground. To test, the effect of such elevated nectar presentation, cotton swabs saturated with 0·5 ml of sucrose solution were displayed at the tip of a 30 cm twig inclined in angles of 0°, 30°, 60° and 90° against the ground level at a distance of 1 m from the nest. The number of ants visiting the cotton swabs in 15 min were recorded.

3.4 *Statistical analysis*

The data on ants per plant and frequency of plants with ants were analysed for regularity, commonness or abundance of ants following the Poisson technique suggested by Williams (1964). The ant diversity was compared by using Shannon-Weaver index.

4. Results

4.1 *Ant composition and their differential visits*

The diversity of ants in the dense zone was higher than that of the sparse zone, and the composition of ant species also differed between dense and sparse zones (table 2). There were no qualitative differences between the composition of the species of ants on the ground and that on the plant; almost all the ant species recovered on the ground were also recovered from the plant, except *Pheidole woodmasoni* which was never recovered from the plants in the sparse zones, though, they were found abundantly on the ground (table 2).

If ant visitation to the plant is directed merely by the presence of nectar, then all the ants recovered from the ground should be represented in corresponding frequencies on the plant also. However, the frequencies of ants on the plants differed from those on the ground in both the zones (figure 1). Figure 2 shows the ratios of percentage of each ant species on the plant and on the ground. These ratios reflect the number of ants of a species found on plant for every ant of the same species found on the ground. Accordingly all the species showing a ratio of more than one can be considered as attracted by the plant and those showing less than one as distracted. Figure 2A shows that 5 species of ants exhibited a ratio of more than 1·5

Table 2. Species of ants found and their diversity on the plant and ground of sparse and dense zones.

Name	Abbre-viations used	Dense zone		Sparse zone	
		Plant	Ground	Plant	Ground
*Meranoplus bicolor** (Guerin)	MB	+	+	+	+
Polyrhachis exercita (Walker)	POL	+	+	−	−
Camponotus sericeus (Fabricius)	CS	+	+	+	+
*Tapinoma melanocephalum** (Fabricius)	TAP	+	+	+	+
Crematogaster sp.	CRMT	+	+	+	+
Solenopsis geminata (Fabricius)	SOL	+	+	+	−
Camponotus compressus (Fabricius)	CC	+	+	+	+
Camponotus rufoglaucus (Jerdon)	CR	+	+	−	−
*Pheidole woodmasoni** Forel	PHI	+	+	−	+
*Monomorium indicum** Forel	MI	−	−	+	+
Diversity index		0·7449	0·6998	0·3127	0·5052

*These ants carry seeds.

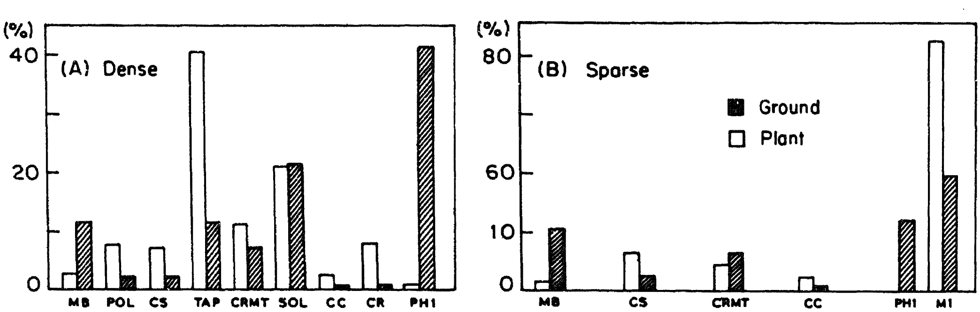


Figure 1. Frequency of different species of ants found on ground and plant in (**A**) dense and (**B**) sparse zones (letters of alphabet refer to species, vide table 2).

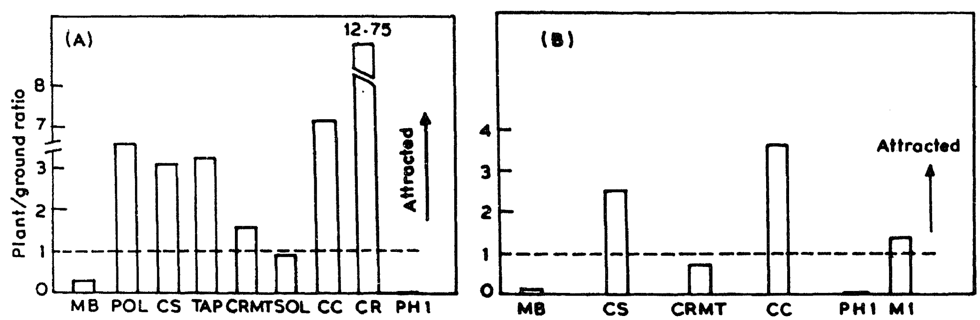


Figure 2. Ratio of frequency of ants on plant to that on ground of different species of ants in (**A**) dense and (**B**) sparse zones (letters of alphabet refer to species, vide table 2).

and two species less than 0·5 in dense zone. Similar differences were also observed in sparse zone. *Camponotus sericeus* and *C. compressus* have shown increased attraction to the plants in both the zones, while *Meranoplus bicolor* and

P. woodmasoni exhibited decreased attraction to the plant. These results indicate that the ant species differ in their visitation to the plant, or the plant exhibits differential attraction of the ant species.

Figure 3A provides a relative classification of ant species as regular or irregular and abundant or rarely available on plants. The ants differed considerably in these features. For instance, *Tapinoma melanocephalum* occurred irregularly, but in abundant numbers in the dense zones while *Camponotus sericeus* was common and regular. In sparse zone *M. indicum* occurred regularly and abundantly while *P. woodmasoni* was rare (figure 3B).

4.2 *Plant factors influencing ant visits*

Table 3 summarises the foregoing results for 4 selected ant species. Some species are attracted by the plant, while some are distracted. They also differed in their visit patterns. These differences can either be due to the plant features and/or due to the innate behavioural features of the ants. We tested the involvement of these factors using a few representative ant species (table 3). Though, it would be difficult to list all the plant features that affect the ant visit, we tested the involvement of (i) pattern of nectar availability on the plant, and (ii) the age of the plant in relation to the nectar quantity.

4.2a *Availability of nectar*: The individual and group foragers differ in their response to the resource quality of the foraging area (Davidson 1977). Utilising the

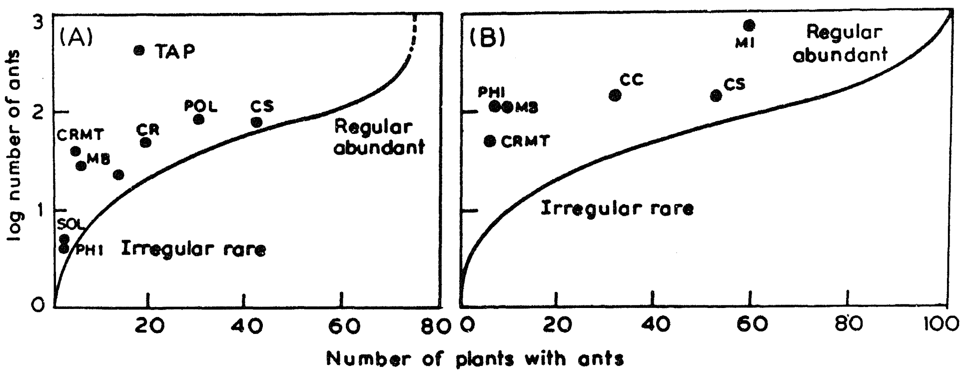


Figure 3. Classification of ant species as rare or abundant, and regular or irregular in (**A**) dense and (**B**) sparse zones (letters of alphabets refer to species, vide table 2).

Table 3. Consistency of visits and abundance of 4 selected species of ants on *C. bonplandianum*.

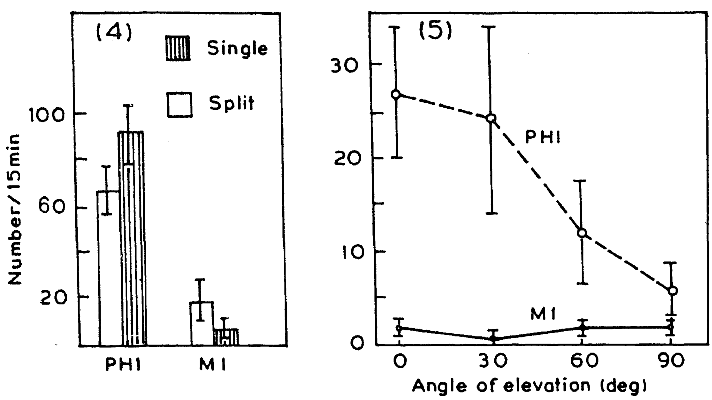
		Visitation features		
Species	Area	Consistency	Abundance	Attraction
Tapinoma melanocephalum	Dense	Irregular	Abundant	Attracted
Monomorium indicum	Sparse	Regular	Abundant	Feebly attracted
Camponotus sericeus	Dense and sparse	Regular	Common	Attracted
Pheidole woodmasoni	Dense and sparse	Irregular	Rare	Distracted

feed back information of the scouts, group foragers tend to visit the resource spots which pay high dividends to the cost expended in harvesting. Hence, they can be expected to preferentially harvest the rich resource spots. The nectar in Croton is presented at 15–45 cm above the ground in split units of 5–1000 small nectaries at the bases of fruits. Each nectary produces 12–40 μg of glucose/h. We hypothesized that such a split pattern of nectar availability might cause differential attraction of group and individual foragers. Figure 4 shows that the total number of *P. woodmasoni* decreased considerably from 92 (in a single unit nectar presentation) to 63 (when the same quantity of nectar was in split units). On the other hand, *M. indicum* showed increased visits from 4·5 in a single unit presentation to 18·63 when presented in split units. Thus the split availability of nectar elicits differential response among the ants.

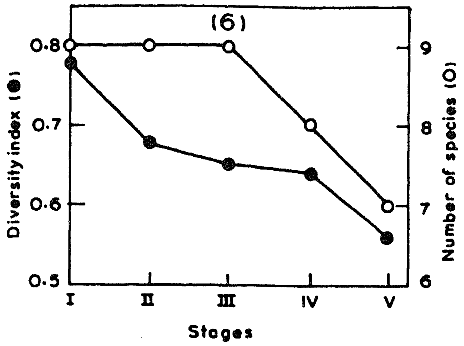
Figure 5 shows the differential ability of the ants to harvest the nectar at varied slopes. *M. indicum* did not differ in harvesting the nectar when available at different levels of inclination, while *P. woodmasoni* preferred to harvest the nectar available at lower inclinations and avoided the nectar with steep inclinations. The dispersed pattern of nectar availability in small units on the vertically oriented racemes of Croton, selectively eliminated *P. woodmasoni* from harvesting it, while *M. indicum* remained unaffected.

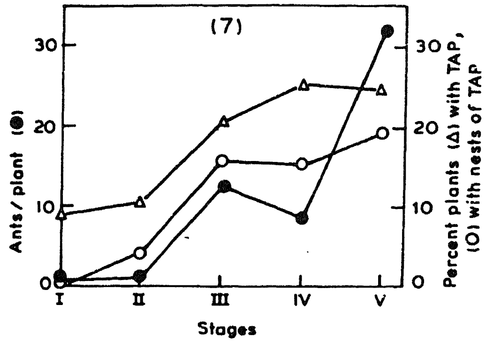
4.2b *Age of the plant and nectar quality*: Diversity index of ants decreased from 0·7769 in the first stage to 0·5647 in the last stage (figure 6). These differences can be explained by the behavioural features associated with the ants and changes in nectar quantity with the stage.

Since the height of the plant increases with age *P. woodmasoni* which tends to avoid harvesting the nectar from steep slopes reduced its visits to the aged plants. On the contrary, the percentage of plants from which *T. melanocephalum* was recovered increased with the stage of the plant (figure 7). Besides, the percentage of the plants under which *T. melanocephalum* nests were recovered also increased with the plant stage. Along with this, there was also an increase in the number of *T. melanocephalum*. Being a nomadic ant, *T. melanocephalum*, shifts its nests very frequently to the sites where resource supply is stable (Ali 1981).



Figures 4 and 5. **4.** Effect of split and single unit presentation of nectar on *Pheidole* (PHI) and *Monomorium* (MI). **5.** Effect of elevation of nectar sources at different gradients on *Pheidole* (PHI) and *Monomorium* (MI).





Figures 6 and 7. 6. Shannon-Weaver diversity index and number of ant species recovered on plants of different growth stages. 7. Frequency of *Tapinoma* (TAP) on plants of different stages of growth and percentage of plants occupied by its colonies.

As the plant ages, the number of nectaries increases (table 1). Simultaneously, because of asynchrony in fruit set between the stages, the nectaries of the different stages overlap in their function, thus providing a continuous nectar supply. The aged plants, thus, constitute the most guaranteed sites of nectar supply encouraging *T. melanocephalum* to occupy them. These ants restrict their foraging area to the foliage of the plant under which they nest. Hence they were recovered only from a small proportion (0·25) of the plants in abundant numbers as the whole colony feeds on the same plant. This explains their abundant, though irregular visit pattern.

5. Discussion

The results indicate that *C. bonplandianum* exhibits differential attraction of ant species. This could be explained by the differences in the behavioural responses of ants to patterns of availability and amount of nectar in a plant. Involvement of the plant characters in regulating ant visits to higher plants is well known (Heads and Lawton 1984; Lawton and Heads 1984; Schemske 1982). For example, the height of inflorescence in *Costus* sp. determines the relative composition of arboreal and terrestrial ant species (Schemske 1982). The tendency of *M. indicum* to be arboreal than *P. woodmasoni* was found to affect their composition on Croton plants.

Since *C. bonplandianum* offers nectar in split units, visits of individual foragers were favoured against group foragers. Such differential response of group and individual foragers of ants to varied food densities has been reported by Davidson (1977). A comparison of composition of individual, intermediate, and group foragers on the ground with that on the plant indicated that plant attracted a greater proportion of individual foragers compared to group foragers (table 4). Such preferential attraction of individual foragers could be beneficial to the plant in dispersing the seeds because these ants are more likely to encounter the seeds on ground due to their tendency to move randomly (Davidson 1977). Ganeshaiah and Umashanker (1988) have observed that individual foragers like *M. indicum* are involved in greater seed dispersal than group foragers. However, they did not test for any correspondence between the frequency of an ant species on plant with its seed

Table 4. Frequency of ants of different types of foraging on *C. bonplandianum* and ground.

Type of foraging	Ground (%)	Plant (%)
Individual	5·27	24·72
Intermediate	11·51	2·48
Group	83·20	62·85

dispersing ability; some ants like *Camponotus* spp. which do not disperse seeds were more regular than seed dispersing ants like *P. woodmasoni.* In other words, the ant-Croton relationship for seed dispersal seems to be a weak mutualism (Koptur 1984). This is evident from that the major seed dispersing ants like *M. indicum* are zone specific and are not always associated with Croton. The habitat differences in ant composition on plants as observed in the present study appears to be a common feature for all facultative ant-plant mutualistic relations (Bentley 1976).

In conclusion, the ant assemblage on *C. bonplandianum* appears to be affected by (i) the habitat and plant density, (ii) stage of the plant and the consequent increase in amount of nectar, and (iii) the pattern of nectar availability, though we cannot negotiate any stronger evidence, towards the evolution of specialised traits in this relationship.

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Byssal threads of *Mytilopsis sallei* (Recluz) and their adhesive strength

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Abstract. The dreissenid mussel *Mytilopsis sallei* (Recluz) settles on man made structures in confined harbour waters. The mussel unlike some mytilids is never found to have settled on coastal sea bed. It shows a good capacity to produce a byssus apparatus and develops a new one every time it settles afresh. Younger mussels develop byssus apparatus at shorter intervals and therefore move more often. Adults are relatively passive. Byssal thread development is influenced by the environmental factors and also by the quality of surface. The mussel achieves better adhesion on polar surfaces like slate and glass than on teflon. Tensile strength of adhesive threads is very poor as compared to other mytilids. This possibly is one of the reasons for its absence on sea beds where sea water turbulence is very high.

Keywords. Marine; biofouling; mytilids; byssal adhesion.

1. Introduction

Mussel *Mytilopsis sallei* has almost established itself in Indian tropical waters. This mussel is not found in open channel waters of Bombay and seems to prefer man-made structures in confined water of harbours. If this mussel can settle or not on the near shore sea bed is yet unknown. In wet basins a fouling debris weighing as much as $10\,kg/m^2$ is generated as a consequence of heavy growth of *M. sallei* (figure 1).

M. sallei has excellent capacity to produce byssus threads and as many as 1000 threads are counted from a single byssus apparatus of an adult mussel pulled off from a submerged surface. The mussel in search of a desired surface can voluntarily shed its byssal apparatus (Udhayakumar and Karande 1986) and develop a new one every time it settles afresh.

Gross morphology of the byssal apparatus of this mussel conforms to that of most of the other mytilids (Bairati and Vitellaro-Zuccarello 1974a, b; Brown 1952). It consists of 3 portions viz. root embedded in the tissues of the foot, a stem and a number of byssus threads each ending in an adhesive disc (figure 2). It is presumed that the adhesive discs in this mussel are amongst the smallest in surface area (0·21 sq.mm). Karande and Menon (1975) observed that *M. sallei* can withstand extreme salinity of 2‰ as long as 4 months of continuous exposure and show seemingly normal activity up to 38°C during 24 h exposure. They have also noted that the mussel survives as long as 10 months on a single dietary source of *Dunaliella primolecta* under laboratory conditions.

Presuming that *M. sallei* is a calm water inhabitant, its reported migration from the Central Atlantic to the Indian Ocean (Morton 1981), is an interesting dispersal phenomenon.

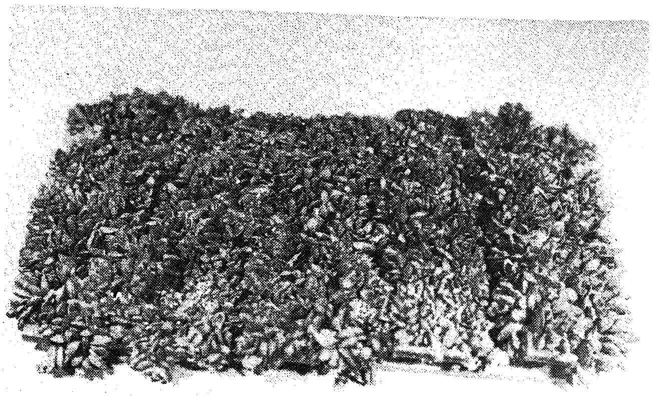

Figure 1. Heavy growth of *M. sallei* in confined polluted waters in Bombay harbour (120 days).

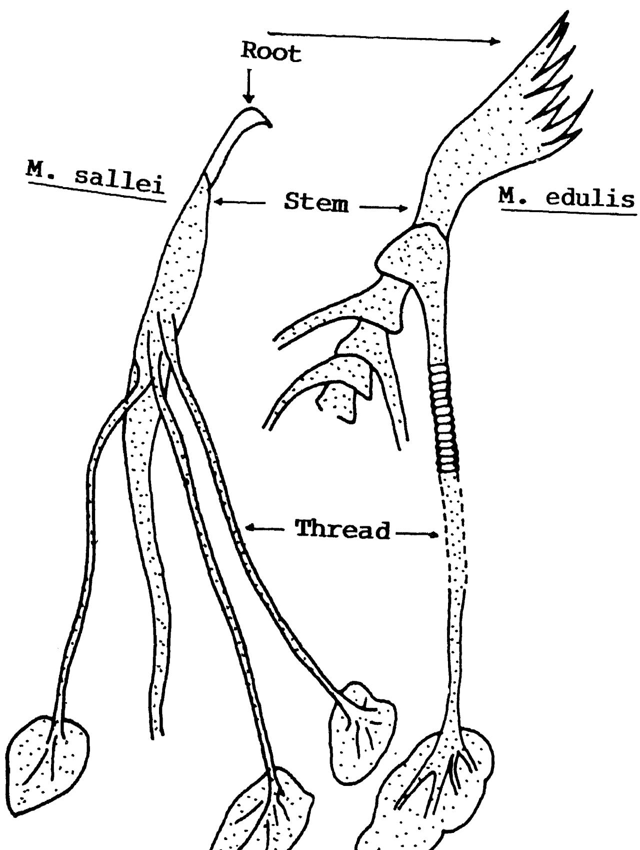

Figure 2. Byssus apparatus of *M. edulis* (after Young and Crisp 1982) and *M. sallei*.

2. Materials and methods

The mussels were collected from the water front structures for the field study and from the fouling coupons for the laboratory experiments. In the laboratory, organisms were kept in an aerated aquarium tanks at 28°C temperature and fed on algal diet of *D. primolecta*.

For counting byssus threads, the mussels were carefully dissected to separate the byssus apparatus. The numbers of threads were counted under stereomicroscope.

The behaviour of mussels on various offered surfaces was examined in 4 litre glass aquaria. Seawater aged for 48 h was used and the mussels were not fed during the experimental period.

For determining adhesive strengths of the byssus apparatus, a method recommended by Gubbay (1983) was adopted. The mussels attached on various surfaces were loaded statically on an Instron tensile testing machine. The load was applied at 20 mm/min and the maximum load borne prior to catastrophic failure resulting in complete detachment of the mussel from the substratum was recorded.

The details of various experiments performed are mentioned alongwith the observations noted under various sections.

3. Results

3.1 *Byssal thread production*

Among the sessile marine organisms, the species securing attachment by byssus threads are at advantage as compared to the others like balanids and polychaetes whose adherence to the surface is permanent. Mytilids can move from place to place if subjected to environmental stresses. It is, therefore, observed that these organisms produce varying number of byssus threads and the adhesive strengths of the byssus vary seasonally and with the nature of environments (Pieters *et al* 1978; Price 1980, 1982; Waite 1983).

3.1a *Field observations*: Table 1 summarises the data on the extent of byssus threads produced at various size classes of the mussels collected from the study

Table 1. Size dependent variations in numbers of byssal threads.

Observations		Size classes in (mm) shell length 10	15	20	>21
Set I	n	21	33	49	15
	$\bar{x}$	141·9 ± 22	159·0 ± 16·4	329·1 ± 31·4	318·0 ± 82
Set II	n	16	52	31	12
	$\bar{x}$	71·0 ± 10·9	132·4 ± 11	202·2 ± 20·3	309·5 ± 34·7
Set III	n	38	45	42	23
	$\bar{x}$	104·1 ± 8·4	163·2 ± 11·7	245·0 ± 12·9	328·0 ± 25·2

n, Number of observations; $\bar{x}$, average number of byssus threads formed.

station. It is seen from this table that the number of threads produced is more in the larger organisms. For instance in set I, comprising 3 independent observations, average numbers of threads counted in each size classes 1, 2, 3 and 4 have been 141, 159, 329 and 318 respectively. A similar trend is noted in sets II and III observations as well.

Field data were further analysed to ascertain if there existed any seasonal influence on the thread production. It is seen from table 2 that the number of threads produced during the monsoon was always numerically more than those produced during pre and post monsoon months. The threads produced during the monsoon were significantly more than those produced during post monsoon period.

3.1b *Laboratory observations*: Table 3 summarises the observations made on the ability of the mussel to produce byssal threads during 120 h under ambient laboratory conditions. Seven to ten individuals of each of 3 size classes, i.e. 5, 15 and 20 mm were used. It was observed that within 120 h the mussels dislodged and resettled on as many as 9 occasions, each time producing varying number of byssus threads. Average numbers of threads produced generally increased with the size class but having reached a size of more than 20 mm, ability to produce fresh byssus threads at consecutive resettlements was reduced. It was evident, therefore, that the younger mussels which displayed better ability to produce threads moved more often than the adult mussels. The latter because of their inability to produce adequate threads at shorter intervals, displayed restricted movements. The adults lack capacity to produce fresh byssal apparatus, but once having properly settled in the field conditions produce larger number of threads than the younger mussels.

3.2 *Salinity and byssus formation*

In these experiments influence of salinity on the development of byssal threads was examined. The surfaces offered for the attachment were gravel, glass and the mussel shells. Five mussels were exposed to each of 10, 20, 35 and 40‰ salinities for a period of 7 days where they often changed places of attachment..

Table 4 gives average numbers of byssus threads formed under different salinity conditions, all the other conditions remaining the same as the ambient laboratory

Table 2. Seasonal variations in the development of byssal threads.

Season	Size class (i) <15 mm (ii) >15 mm	'P' Value	Statistical analysis: Season	Statistical analysis: Remarks
Monsoon	(i) 147 ± 12	0·05	Monsoon/Post-monsoon	S
	(ii) 325 ± 27	0·02		
Post-monsoon	(i) 117 ± 9	0·10	Post-monsoon/Pre-monsoon	NS
	(ii) 238 ± 19	0·20		
Pre-monsoon	(i) 141 ± 9	0·50	Monsoon/Pre-monsoon	NS
	(ii) 274 ± 12	0·10		

S, Significant; NS, not significant.

Table 3. Byssal thread production under laboratory conditions.

Size class (mm)	Specimen	No. of byssus produced at every fresh attachment during 120 h									Cumulative no. of threads	Remarks
		1	2	3	4	5	6	7	8	9		
5	1	3	9	20	60	—	—	—	—	—	92	Av. cumulative/individual = 66 ± 12·9
	2	5	10	16	11	13	18	6	18	25	122	
	3	8	25	—	—	—	—	—	—	—	33	
	4	7	11	13	20	18	7	—	—	—	76	
	5	4	7	11	23	—	—	—	—	—	45	
	6	6	7	10	10	—	—	—	—	—	33	
	7	4	4	7	11	5	—	—	—	—	31	
	8	10	7	6	6	8	—	—	—	—	37	
	9	4	21	16	8	16	8	46	6	—	125	
15	1	9	15	15	25	41	19	6	—	—	130	Av. cumulative/individual = 92·85 ± 19·9
	2	25	42	38	12	26	12	—	—	—	155	
	3	8	6	6	—	—	—	—	—	—	20	
	4	6	18	34	14	19	29	20	9	—	149	
	5	2	12	12	9	14	22	9	—	—	80	
	6	36	7	—	—	—	—	—	—	—	43	
	7	21	20	32	—	—	—	—	—	—	73	
20	1	31	—	—	—	—	—	—	—	—	31	Av. cumulative/individual = 18·14 ± 4·36
	2	24	13	—	—	—	—	—	—	—	37	
	3	1	6	11	—	—	—	—	—	—	18	
	4	10	—	—	—	—	—	—	—	—	10	
	5	13	—	—	—	—	—	—	—	—	13	
	6	12	—	—	—	—	—	—	—	—	12	
	7	6	—	—	—	—	—	—	—	—	6	

Table 4. Byssus threads produced under various salinity conditions.

	Salinity (‰)			
	10	20	35	40
No. of threads (Cumulative of 5 mussels)	404 ± 28	330 ± 45	278 ± 24	229 ± 53
Influence of salinity	10/35 salinity $P < 0.005$ S	20/35 salinity $P < 0.50$ NS		35/40 salinity $P < 0.50$ NS

S, Significant; NS, not significant.

environment. Assuming 35‰ as a prevailing normal sea water salinity, an increase in number of threads was noted in organisms exposed to lowest salinity of 10‰. There were no significant variations in numbers at salinities 20 and 40‰.

3.3 *Temperature and byssus formation*

The mussels were subjected to 4 different temperatures of 15, 20, 30 and 35°C. It was observed (table 5) that there was no significant influence of raised temperature on the thread formation. At lower temperatures of 15 and 20°C, however, the numbers of threads formed were significantly small than those formed at ambient temperature of 30°C.

3.4 *Substrate quality and attachment*

3.4a *Mussel behaviour on independent surfaces*: The preference of the mussel for 2 polar surfaces namely slate and glass and a non-polar teflon was examined. The mussels also opted to settle on the shells of the fellow experimental organisms and therefore the settlement on this surface was also considered in these experiments. Each experiment was carried out for a period of 3 weeks continuously.

Three groups of mussels identified as (a), (b) and (c), each containing 15 mussels were placed on each of the 3 surfaces by rotation for a period of one week. For instance (see table 6), group (a) mussels were placed on slate in the first week, then on glass in the second week and on teflon in the third week. In each group there had been a steady fall in thread making activity during each week. It is for this reason that as recommended by Young (1983) 3 groups had to be used and placed in turn on each surface. Table 6 shows that on slate, glass and teflon more threads were formed than on mussel shell. Table 6 (row 7) gives ratios of numbers of discs formed on offered surfaces to number of discs on shells. All the 3 surfaces, slate, glass and teflon, in that order were preferred to shell surface.

3.4b *Choice of surface for byssal attachment*: Three pairs of surfaces like slate/glass, wax/slate and glass/wax were offered to examine mussels choice for the surface. Eight mussels were placed on each surface and the experiment was repeated for 5 weeks, each week mussels being subjected to different choice between pairs of surfaces.

Table 5. Byssus threads produced under various temperature conditions.

	Temperature (°C)			
	15	20	30	35
No. of threads (cumulative of 5 mussels)	$7{\cdot}6 \pm 1{\cdot}8$	$46{\cdot}8 \pm 9{\cdot}7$	$208{\cdot}1 \pm 21{\cdot}7$	$171{\cdot}7 \pm 25{\cdot}6$
Influence of temperature	15/20°C $P < 0{\cdot}0005$ HS	20/30°C $P < 0{\cdot}00005$ HS		30/35°C $P < 0{\cdot}05$ NS

HS, Highly significant; NS, not significant.

Table 7 shows that in the 5 weekly trials, the numbers of discs formed on wax were always smaller than those on glass and slate. It is also observed that the numbers of pads per unit area of surface were always greater by several order of magnitude on the polar surfaces of slate and glass.

3.4c *Surface texture and byssal deposition*: To assess the influence of surface texture on the deposition of byssal threads, 7 surfaces were offered for the settlement. Three of these were roughened and offered with their smooth counterparts. Table 8 shows that glass, fibreglass and perspex when roughened, induced higher thread formation as compared to their smooth surfaces.

3.4d *Byssal deposition on gravel*: An experimental set up for carrying out this experiment is illustrated in figure 3. It is seen from table 9 that the mussels not only preferred gravel surface to teflon but in doing so produced more adhesive discs on gravel. Some mussels adhered partly to gravel and partly to teflon but even in this event more discs were adhered to the former.

3.4e *Surface quality and disc area*: Twenty mussels of varying shell sizes were allowed to settle on glass surface. Figure 4A shows a relationship between mean area of discs formed by each individual and its shell length. The disc area was found to grow linearly with the increasing shell size.

Ten mussels each were allowed to grow on polar and non-polar surfaces for a period of 10 days. Average areas of discs of the mussels settled on slate, glass, perspex and teflon were 0·13, 0·21, 0·26 and 0·16 mm^2. No correlation therefore was noted between disc area and the polarity of the surface.

3.5 *Adhesive strength of byssal attachment*

In order to measure adhesive strength of the mussel, a method earlier adopted by Gubbay (1983) was followed. Figure 4B, C shows the relationship between the areas covered by discs on different surfaces and the forces required for the dislodgement of the individual mussels. There appears to be no relation between the two. It is inferred therefore that though the disc area increases with the growth of the shell, an adhesive bond secured by individual mussel is not proportional to the area of adhesion achieved. This implies that the discs lose adhesive bond as they grow old, necessitating continuous production of fresh threads by this mussel.

Table 6. Number of newly formed threads and location of attachments of pads on different surfaces.

Surface	Slate			Glass			P.T.F.E.			Total no. of threads
No. of pads	On slate I	On mussel II	Total III	On glass IV	On mussel V	Total VI	On P.T.F.E. VII	On mussel VIII	Total IX	On all surfaces (% on mussels) X
Week 1	(a) 582	92	674	(b) 232	0	232	(c) 219	148	367	1273 (18·85)
2	(c) 394	102	496	(a) 221	120	341	(b) 439	107	546	1383 (23·78)
3	(b) 136	14	150	(c) 257	27	284	(a) 118	23	141	575 (11·13)
Total	1112	208		710	147		776	278		
Ratio of no. of pads on surface to nos. on mussels	5·34:1			4·82:1			2·79:1			

Table 7. Numbers of pads formed on 3 pairs of surfaces slate/glass, wax/slate and glass/wax during 5 successive weeks.

Week	Pairs of surface	No. of pads	Pads/cm^2
1	Slate and glass	7 and 186	0·02 and 0·96
	Wax and slate	27 and 276	0·11 and 1·40
	Glass and wax	182 and 1	0·94 and 0·004
2	Slate and glass	130 and 36	0·53 and 0·18
	Wax and slate	7 and 73	0·02 and 0·37
	Glass and wax	24 and 1	0·12 and 0·004
3	Slate and glass	92 and 82	0·38 and 0·42
	Wax and slate	9 and 76	0·03 and 0·39
	Glass and wax	291 and 1	1·5 and 0·004
4	Slate and glass	169 and 62	0·69 and 0·3
	Wax and slate	5 and 313	0·02 and 1·29
	Glass and wax	174 and 4	0·9 and 0·01
5	Slate and glass	7 and 30	0·02 and 0·15
	Wax and slate	1 and 167	0·004 and 0·86
	Glass and wax	138 and 17	0·76 and 0·07

Table 8. Byssal threads produced during 7 days under laboratory condition.

	No. of threads Surface condition	
Surface	Smooth	Rough
Slate	32·5 ± 6	—
Asbestos	31·0 ± 6·42	—
Balasa wood	15·0 ± 2·89	—
Glass	12·0 ± 2·41	32·0 ± 11·9
Fibre glass	12·0 ± 4·14	31·0 ± 9·59
Perspex	27·17 ± 4·17	48·42 ± 7·42
Wax	8·0 ± 1	—

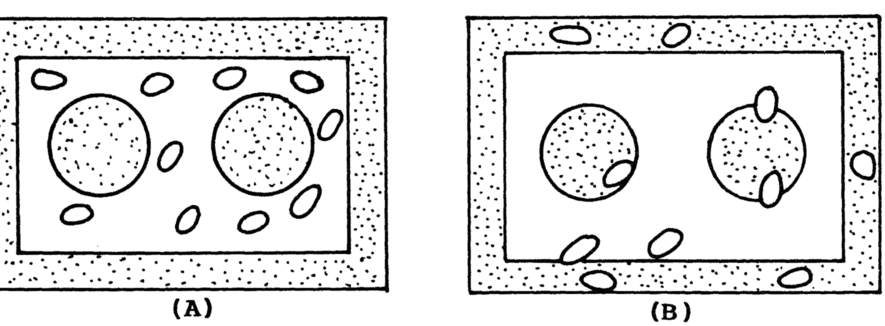

Figure 3. Schematic representation of the experimental set up for ascertaining the choice of *M. sallei* for teflon (total area 1394 sq.cm) and gravel (total area 766 sq.cm) substrates. **A.** Position at the beginning of experiment. **B.** Position at the end of experiment.

igure 4B, C also reveals that the forces required for dislodging the mussels from two polar surfaces namely slate and glass are greater (up to 112 g) than that

Table 9. Choice of *M. sallei* between teflon and gravel surfaces.

Experiment	Gravel: No. of mussel attached	Gravel: Av. no. of thread/ mussel	Teflon: No. of mussel attached	Teflon: Av. no. of thread/ mussel	Preference to gravel: As per mussels attached	Preference to gravel: As per threads formed
1	15	37·8	3	26·6		
2	10	25·7	4	16·5	$t=2{\cdot}79$ $P<0{\cdot}05$ S	$t=2{\cdot}861$ $P<0{\cdot}05$ S
3	11	28·3	5	13·0		
Average	12 ± 1·5	30·6 ± 3·6	6·3 ± 1·3	18·7 ± 4·0		

S, Significant.

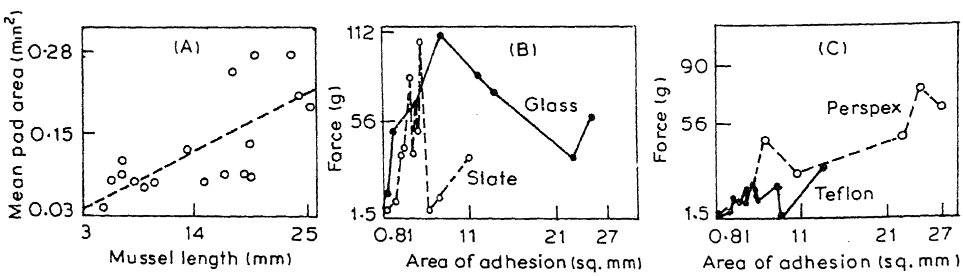

Figure 4. Relationship between (**A**) *M. sallei* shell length and mean adhesive pad area, (**B**) area of adhesion and the force required for dislodging the mussel from slate and glass surfaces and (**C**) area of adhesion and the force required for dislodging the mussel from perspex and teflon (PTFE) surfaces.

needed for teflon (32 g). Udhayakumar and Karande (1986) have reported that the force required for the removal of *M. sallei* is far less ($0{\cdot}09 \times 10^8$ Nm^{-2}) than that required for big mussel *M. edulis*. In the latter species a force of $3{\cdot}6 \times 10^8$ Nm^{-2} is required to dislodge one single byssal thread (Young and Crisp 1982). The force required to pull off the whole animal therefore is expected to be very high.

4. Discussion

M. sallei on a given surface readily forms byssus within 7 min, the successive threads emerging at intervals of 2 or 3 min. In this respect its ability to secure first attachment is as good as *M. edulis* (Young 1983). The mussel can voluntarily shed its byssus apparatus as often as 6 times within 24 h and 9 times in 120 h. This is an advantageous situation so far as the freedom of the mussel to move and resettle is concerned.

M. sallei produces a larger number of adhesive threads during the monsoon period. It was noted that more threads were formed at lower salinities of 10‰ than at normal saline water. An ability of the mussel to sustain low and widely varying salinity has, as a matter of fact, helped this species to establish itself in Indian waters (Karande and Menon 1975). This mussel produced less number of byssal threads at lower temperatures of 15 and 20°C as compared to normal ambient

temperature of 30°C. Waite (1985) has, however, observed that 'none of these (earlier) studies conclusively demonstrates that byssal formation is specifically influenced by environmental factors, instead, the changes in byssal formation are often used to measure changes in the byssal rate of metabolism'.

No chemo-reception mechanism is studied in these mussels, however, a tuft of cillia on foot in *M. edulis* is described as being mechanoreceptors (Young 1983). Seed (1967) concluded that smooth surfaces such as tufnol attracted very few larvae, the maximum settlement being on roughened or fibrous surfaces. Both Maas Geesteranus (1942) and Chipperfield (1953) made similar observations. Young and Crisp (1982) inferred that 'as far as possible mussels avoid attaching the byssus to low energy surfaces, and do so only when high energy surfaces are not made available'.

In *M. sallei* it was noted that rough surface did induce higher development of byssal threads. It was observed that the thread formation on non-polar wax surface was significantly poor as compared to slate or glass surface. In *M. sallei*, the shell was found to be a least preferred substrate as was a teflon surface.

Earlier Udhayakumar and Karande (1986) had observed that in *M. sallei* there was no correlation between the disc areas and polarities of the offered surfaces. They, however, observed that the forces required to pull off the mussel from glass and slate were greater (up to 112 g) than that needed for teflon (32 g).

M. sallei are found to have ability to settle on gravel but this by itself is not adequate to maintain a sustained adhesion to the sea-bed. Besides smaller adhesive discs ($0{\cdot}21\ mm^2$), a smaller body size and a shorter foot, one more factor that possibly limits the distribution of *M. sallei* in open waters is its inability to sustain a relatively high turbulence earlier reported by Swami and Karande (1988). The tensile strength of byssal complex of *M. sallei* as a whole is around $0{\cdot}09 \times 10^8\ Nm^{-2}$ (Udhayakumar and Karande 1986). On the other hand the strength of one single byssus thread of *M. edulis* is $3{\cdot}6 \times 10^8\ Nm^{-2}$. Despite this superior tensile strength of the byssal thread of *M. edulis*, Price (1982) observes that in that species 'wave action emerges as a major determinant of byssal attachment strength'. This could as well be true in case of *M. sallei*.

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Effect of feeding tender and senescent leaf by *Eupterote mollifera* and tender leaf and flower by *Spodoptera exigua* on food utilization

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Abstract. Food consumption and utilization efficiencies of *Eupterote mollifera* fed on tender leaf were higher than those of counterparts ingesting senescent leaf, while in *Spodoptera litura* individuals fed on flower showed higher values in comparison to those that consumed tender leaf. Food utilization parameters of these insects depend upon the nutritional quality of the food.

1. Introduction

Reviews of Slansky and Scriber (1985) and Muthukrishnan and Pandian (1987) show food quality and availability remarkably influence rates and efficiencies of food utilization in herbivorous insects. Russel (1947) and Mattson (1980) demonstrated that nitrogen content of plants varies widely not only between different species but also between the different parts of the same plant. Herbivores feeding nutrient rich seed, pollen or nectar display higher efficiencies of assimilation and production than others feeding on leaf or flower (e.g. *Megachile pacifica* feeding on pollen and nectar: $Ase = 88\%$; $Pe_2 = 66\%$: Wightman and Rogers 1978; *Callosobruchus analis* fed on seeds: $Ase = 85\%$, $Pe_2 = 59\%$: Wightman 1978). Senthamizhselvan and Murugan (1988) demonstrated that different levels of nutrients in the host plant leaves not only influence ingestion and transformation of food in *Atractomorpha crenulata* but also its fecundity. Barring the publication of Marian and Pandian (1980) very little is known on the effect of age-dependent changes in nutritional quality of the host plant leaf and food utilization by lepidopterous larvae. The present paper reports food utilization of *Spodoptera litura* fed *ad libitum* on tender leaf and flower of *Brassica oleracea* and of *Eupterote mollifera* fed on tender leaf and senescent leaf of *Moringa olifera*.

2. Materials and methods

Newly hatched larvae of *Spodoptera exigua* and *E. mollifera* were collected from their host plants (*B. oleracea* and *M. olifera*). As soon as the larvae moulted into penultimate instar, they were divided into two groups each. One group of *E. mollifera* was fed on tender leaf and the other on senescent leaf. *S. litura* larvae were fed on flower or tender leaf of *B. oleracea*. As lepidopterous larvae ingest more than 80% of their total food consumption during the penultimate and final instar larval stages (Waldbauer 1968), the present experiment was restricted to these instars only. The fourth leaf from the terminal bud was considered as tender leaf and the wilted, less chlorophyll containing yellowish leaf at the bottom-most part of the stem

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or branch of the plant as the senescent leaf. The larvae were fed *ad libitum* on tender leaf or senescent leaf or flower twice a day. Food consumption (C), assimilation (A), production (P) and metabolism (M) of the larvae were estimated following the gravimetric method of Waldbauer (1968) and the scheme of energy balance followed in the present study is the IBP formula of Petrusewicz and Macfadyen (1970) represented as

$$C = P + R + FU$$

and described in detail elsewhere (Senthamizhselvan and Muthukrishnan 1988). Energy content of the samples of leaf or flower, feces and insect was estimated in a 1421 semimicro bomb calorimeter. Nitrogen content of the sample was estimated in microkjeldahl apparatus following the method of Umbreit *et al* (1974). Considering the energy and nitrogen content of appropriate samples, C, FU and P of the larvae, energy and nitrogen budgets were calculated. Rates of food energy or nitrogen ingestion (Cr) or (NCr), assimilation (Ar) or (NAr), production (Pr) or (NPr) and metabolism (Mr) or residual were calculated following the method described by Muthukrishnan and Pandian (1987). Energy budget for the non-feeding pupal period was prepared following the formulae given below:

$$TL = Ex + Pu + R1$$
$$Pu = Pc + A + R2$$
$$A = E + Ad + R3$$

TL, Pu, A and Ad represent the energy contents of terminal larva, pupa, freshly emerged adult and adult at death respectively. Ex, Pc and E represent the energy allocated to exuvia (final instar), pupal case and egg respectively. $R1$, $R2$ and $R3$ represent energy expended on metabolism by pharate pupa, pharate adult and adult respectively. Metabolic rate of the pharate pupa and pharate adult as well as the adult was calculated by dividing the energy expended on metabolism during the respective stages by the product of mid-body weight (g) and duration (day) (see also Muthukrishnan and Pandian 1984).

3. Results

Table 1 provides data on nitrogen, water and energy contents of the food. Tender leaf of *M. olifera* contained more nitrogen (4·83%) and water (74·71%) than the senescent leaf (2·08%; 50·04%). Nitrogen content of the flower of *B. oleracea* (6·35%) was twice more than that (3·15%) of the tender leaf. Energy content of the insects at the commencement of V instar varied between 391 and 648 J. Despite commencing with low energy content *E. mollifera* accumulated more energy (3431 J) than *S. litura* by extending the duration of the larval period to 10 days; the duration for *S. litura* feeding on flower was 6–6·5 days. Ingestion of senescent leaf resulted in a less significant prolongation of the larval duration and a very highly significant decrease in the energy contents of the terminal larva, pupa and adult. For instance, energy content of terminal larva, pupa and adult of *E. mollifera* in the senescent leaf schedule was 1572, 1336 and 624 J, respectively against 3431, 3088 and 1814 J for those in the tender leaf schedule (figure 1). In about 6·5 days female *S. litura* feeding on flower accumulated 5816 J at the time of completion of larval development, while male completed larval development in 5·9 days and contained 4149 J. Female or

Table 1. Nitrogen, water and energy content of the host plants.

Host plant	Nitrogen (% dry wt)	Water (%)	Energy (J/mg)
M. olifera			
Tender leaf	4·83 ± 0·26	74·71 ± 4·07	20·06 ± 0·80
Senescent leaf	2·08 ± 0·17	50·04 ± 3·30	14·95 ± 0·52
B. oleracea			
Flower	6·35 ± 0·26	80·46 ± 4·10	21·55 ± 0·82
Tender leaf	3·15 ± 0·17	76·31 ± 3·82	18·33 ± 0·59

Each value (X ± SD) represents the average of 4 estimations.

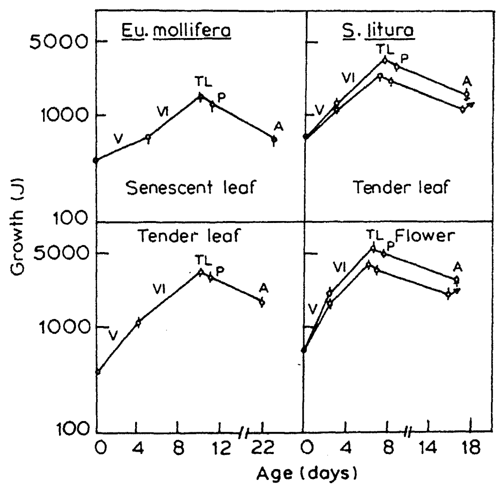

Figure 1. Growth of *E. mollifera* and *S. litura* fed *ad libitum* on tender or senescent leaf or flower of their respective host plant. Roman numerals and vertical lines indicates larval instars and SD respectively.

male *S. litura* feeding on tender leaf for about 7·5 or 7 days accumulated 3475 or 2536 J. At the time of emergence, *S. litura* female or male in the flower feeding schedule contained 2992 or 2097 J; corresponding energy content of the adult in the tender leaf feeding schedule was 1702 or 1170 J.

E. mollifera ingested (16331 J) 44% more food than larva feeding on senescent leaf (8740 J) (table 2). A 2-fold increase in the N content of *B. oleracea* flower over that of tender leaf resulted in about 35% increase in the ingestion of flower by female *S. litura* (table 2). A comparison of feeding rates of these two species indicated that the *Cr* of the larva feeding on tender leaf or flower increased from that feeding on senescent leaf. For instance, *Cr* of *E. mollifera* feeding on tender leaf increased to 5·57 kJ/g/day from 2·758 kJ/g/day of the larvae feeding senescent leaf. However, the increase in *Cr* of *S. litura* male or female feeding on flower over that feeding on tender leaf was only marginal (table 2).

Corresponding to the increase in *C* of the larvae feeding tender leaf, energy loss through *FU* also increased. During the last two instars, *E. mollifera* in the tender leaf schedule lost 7431 J equivalent *FU* compared with 4391 J in the senescent leaf

Table. 2 Overall energy budget for the penultimate and final instar larval stages of the pests fed *ad libitum* on their host plant.

	E. mollifera		*S. litura*			
			Female		Male	
Parameter	Tender leaf	Senescent leaf	Flower	Tender leaf	Flower	Tender leaf
D	10·0	11·0	6·5	7·5	5·9	7·0
C*	16330·8	8740·1	23968·0	15515·8	17756·4	11662·0
FU*	7430·8	4391·0	10511·7	7431·4	8103·5	5720·1
A*	8900·0	4351·0	13456·3	8084·4	9652·9	5941·9
P*	3041·5	1179·9	5164·0	2795·4	3516·3	1906·9
R*	5858·5	3171·1	8292·3	5289·4	6136·6	4035·0
Cr**	5·570	2·758	8·239	5·519	7·720	4·729
Ar**	3·056	1·378	4·659	2·897	4·243	2·419
Pr**	0·991	0·357	1·720	0·995	1·491	0·759
Mr**	2·066	1·021	2·939	1·882	2·752	1·660
Ase(%)	54·5	49·8	56·1	52·1	54·4	51·0
Pe_1(%)	18·6	13·5	21·5	18·0	19·8	16·4
Pe_2(%)	34·2	27·1	38·2	34·6	36·4	32·1

Each value represents the average performance of 10–13 larvae.
D, Day; C, consumption; FU, faeces; A, assimilation; P, production; R, metabolism; Cr, consumption rate; Ar, assimilation rate; Pr, production rate; Mr, metabolic rate; Ase, Assimilation efficiency; Pe_1, Gross production efficiency; Pe_2, Net production efficiency.
*J/larvae; **kJ/g/day.

schedule (table 2). Briefly, feeding water and N rich host plant resulted in the loss of lesser fraction of the ingested energy through FU.

During the last two instars, *E. mollifera* feeding on tender leaf assimilated 8900 J compared with 4351 J feeding on senescent leaf. Remarkable differences were observed between energy assimilated by *S. litura* males and females feeding on flower and tender leaf of *B. oleracea* (table 2). Similar trends were observed for Ar like that of consumption rate. For instance, Ar of *E. mollifera* feeding on tender leaf was 3·387 kJ/g/day compared with 1·260 kJ/g/day for that feeding senescent leaf (table 2). Assimilation efficiency of the larva feeding on tender leaf was significantly higher than that of feeding on senescent leaf. For example, the efficiency of *S. litura* larva feeding on *B. oleracea* flower was significantly higher than that of the larva feeding on tender leaf (table 2). Larvae feeding on tender leaf displayed higher metabolic rate than those feeding on senescent leaf. At an overall rate of 2·066 kJ/g/day *E. mollifera*, feeding on tender leaf metabolised 5859 J of assimilated energy. On the other hand, larvae feeding on senescent leaf metabolised 3171 J at the rate of 1·02 kJ/g/day (table 2). Briefly, irrespective of the life stage or food quality, the larvae expended over 60% of the assimilated energy on metabolism. Larvae feeding on tender leaf registered more than 2-fold increase in production over those feeding on senescent leaf. For instance, *E. mollifera* feeding on tender leaf produced larval matter equivalent to 3042 J compared with 1180 J for those feeding on senescent leaf. Briefly, increase in consumptioh or assimilation owing to the better quality of the food resulted in an increase in production. Net production efficiency of the larvae feeding on flower was 3% more than that feeding tender leaf. Similar trends were observed for the *S. litura* larvae feeding on tender or senescent leaf.

Irrespective of food quality or species, the pharate pupal period lasted for 1 day.

E. mollifera expended about 25 J of the energy accumulated in the terminal larva (*TL*) to exuvia (*Ex*) and about 50 J to silk production and cocoon formation (*Pc*). Briefly, energy loss on final instar *Ex*, and *Pc* appears to be determined by the size of the *TL*. After incurring a loss of 343 and 236 J on structural and functional components, *TL* of *E. mollifera* belonging to the tender and senescent leaf schedules metamorphosed into pupa with 3088 and 1336 J. Energy content of female *S. litura* pupa in the flower or tender leaf schedule was 5234 or 2996 J, while corresponding value for the male was 3692 or 2155 J (table 3). In the process of transformation of pupa into adult, more than 40% of the energy was lost on *Pc* and maintenance metabolism. The efficiency with which pupal matter was transformed into adult (pupal efficiency) was higher in the tender leaf feeding schedule than in the senescent leaf schedule. A critical analysis of the metabolic rates of pharate pupa and pharate adult of these pests revealed that the rates were higher in the tender leaf schedule and flower schedule and the rate for the male *S. litura* was higher than that of the female in both the schedules. The energy cost of metamorphosis ranged from 184 J/g/day to 514 J/g/day. *S. litura* feeding on flower or tender leaf expended 557·3 J (453 egg) or 259·3 J (211 egg) on egg production. Egg production efficiency of *S. litura* ranged between 15·2 and 18·6% (table 3).

Trends obtained for nitrogen ingestion by the larvae were similar to those

Table 3. Energy budget of pharate pupa and pharate adult of *E. mollifera* and *S. exiqua.*

	E. mollifera		*S. litura*			
			Female		Male	
Parameter	Tender leaf	Senescent leaf	Flower	Tender leaf	Flower	Tender leaf
*TL**	3430·7 ± 183·4	1572·0 ± 84·3	5816·0 ± 288·6	3475·4 ± 182·3	4148·7 ± 207·3	2535·5 ± 140·8
PPD	1·0 ± 0·0	1·0 ± 0·0	1·0 ± 0·0	1·0 ± 0·0	1·0 ± 0·0	1·0 ± 0·0
Exuvia*	26·4 ± 1·3	24·8 ± 1·2	23·7 ± 1·1	22·0 ± 0·9	22·0 ± 0·9	20·4 ± 0·8
Silk*	52·5 ± 2·8	48·3 ± 3·6	—	—	—	—
Pupa*	3087·6 ± 169·6	1336·2 ± 72·3	5234·4 ± 280·5	2994·8 ± 162·4	3692·3 ± 199·8	2155·2 ± 166·8
R1*	264·2 ± 15·8	162·7 ± 9·8	558·6 ± 32·3	438·6 ± 24·6	434·3 ± 22·1	359·9 ± 19·4
PAD	9·0 ± 0·5	9·0 ± 0·5	9·0 ± 0·5	9·0 ± 0·5	9·0 ± 0·5	9·0 ± 0·5
Adult*	1813·9 ± 98·7	624·0 ± 34·8	2991·5 ± 171·4	1701·6 ± 91·9	2097·2 ± 114·5	1170·3 ± 64·2
*Pc**	43·4 ± 2·6	40·1 ± 2·2	53·6 ± 2·9	44·3 ± 2·0	51·4 ± 2·8	43·8 ± 2·2
*R2**	1230·3 ± 67·5	672·1 ± 37·4	2189·3 ± 130·4	1249·9 ± 67·3	1543·7 ± 82·9	941·1 ± 51·6
PE(%)	58·7 ± 3·1	46·7 ± 2·4	57·2 ± 3·0	56·8 ± 2·8	56·8 ± 2·8	54·3 ± 2·4
Egg*	—	—	557·3 ± 32·1	259·3 ± 14·9	—	—
EPE(%)	—	—	18·6 ± 1·0	15·2 ± 1·0	—	—
*AD**	808·6 ± 44·7	312·4 ± 17·8	885·6 ± 47·7	507·1 ± 27·9	199·2 ± 56·4	555·9 ± 30·4
ADU	3·5 ± 0·5	3·5 ± 0·5	3·5 ± 0·5	3·5 ± 0·5	3·0 ± 0·5	3·0 ± 0·5
*R3**	961·9 ± 52·7	271·5 ± 14·7	1495·0 ± 84·0	890·9 ± 49·4	1026·6 ± 56·8	570·6 ± 31·1
*PPMr***	370·0 ± 20·2	312·4 ± 18·4	724·5 ± 39·6	695·5 ± 39·4	779·6 ± 42·4	767·3 ± 42·1
*PAMr***	264·4 ± 16·8	209·9 ± 12·7	499·0 ± 27·4	335·5 ± 20·1	513·5 ± 28·6	394·9 ± 22·4
*AMr***	1876·0 ± 101·6	852·2 ± 48·1	2366·4 ± 132·1	1858·0 ± 99·8	2061·7 ± 111·0	1811·4 ± 97·4
*MMr***	244·7 ± 14·2	184·1 ± 12·9	455·2 ± 25·2	350·8 ± 20·1	483·4 ± 26·1	367·7 ± 19·8

Each value (X ± SD) represents the average performance of 10–13 insects.

TL, Terminal larva; PPD, pharate pupal duration (day); R1, pharate pupal metabolism; R2, pharate adult metabolism; R3, adult metabolism; PAD, pharate adult duration (day); Pc; pupal case; PE, pupal efficiency; EPE, egg production efficiency; Ad, adult at death; ADU, adult duration (day); PPMr, pharate pupal metabolic rate; PAMr, pharate adult metabolic rate; AMr, adult metabolic rate; MMr, metamorphic metabolic rate. *J/insect; **J/g live insect/day.

reported for the ingestion of food energy. At an overall rate of 13·423 and 11·691 or 8·888 mg/g/day, *E. mollifera* and *S. litura* female or male belonging to tender leaf feeding schedules ingested 39·339 and 28·451 or 21·443 mg nitrogen respectively (table 4). About 10–40% of the ingested nitrogen was lost through *FU* by the larvae in the different feeding schedules. The nitrogen assimilation rate (*NAr*) of *E. mollifera* feeding tender leaf (11·808 mg/g/d) was 6 times more than the corresponding rate of the larvae feeding on senescent leaf (2·011 mg/g/d). Nitrogen assimilation efficiency (*NAse*) depended on N content of the food. The efficiency ranged between 52·4 and 90·1%. At an overall rate of 9·64 and 6·279 or 4·847 mg/g/d, *E. mollifera* and *S. litura* female or male feeding on tender leaf accumulated 28·368 and 17·624 or 12·041 mg nitrogen respectively (table 4).

4. Discussion

Under conditions of extreme defoliation of host plant, lepidopterous larvae may be forced to consume leathery, nutritionally poor senescent leaf (Marian and Pandian 1980). A comparison of N, water and energy contents of tender and senescent leaf of *M. olifera* showed that the senescent leaf is nutritionally inadequate. Ingestion of nutritionally inadequate leaf produced several negative effects like decrease in the final body weight or energy content of the terminal larva and adult, larval and pupal mortality, and significant reduction in rates and efficiencies of food utilization in *E. mollifera*. Similar negative effects have been reported by Marian and Pandian (1980) for *Danaus chrysippus* fed on senescent leaf of *Calotropis gigantea*. *Cr* of *E. mollifera* fed on senescent leaf was about 50% of those fed on tender leaf. The reduction in *Cr* and *NCr* was apparently due to about 35% decrease in the water content and 40–57% decrease in the N content of the senescent leaf. However, the response of the chrysomelid beetle *Phaedon cochlearriae* feeding less N containing 8 week old turnip leaf was quite different. The beetle feeding 49% less

Table 4. Overall nitrogen budget for the penultimate and final instar larval stages of the pests fed *ad libitum* on their host plant.

	E. mollifera		*S. litura*			
			Female		Male	
Parameter	Tender leaf	Senescent leaf	Flower	Tender leaf	Flower	Tender leaf
D	10·0	11·0	6·5	7·5	5·9	7·0
*C**	39·339	12·183	70·578	28·451	52·287	21·443
*FU**	5·774	5·801	6·958	6·808	5·275	5·241
*A**	33·565	6·382	63·620	21·643	47·012	16·202
*P**	28·368	4·826	53·697	17·624	36·531	12·041
*r**	5·197	1·556	9·923	4·019	10·490	4·161
*NCr***	13·423	3·845	24·016	11·691	18·657	8·888
*NAr***	11·808	2·011	23·122	8·853	17·190	6·782
*NPr***	9·640	1·504	17·608	6·279	12·514	4·847
NAse(%)	85·32	452·38	90·1	76·1	89·9	75·6
NPe_1(%)	72·11	39·61	76·1	61·9	69·9	56·2
NPe_2(%)	84·52	75·62	84·4	81·4	77·7	74·3

Each value represents the average performance of 10–13 larvae.
See table 2 for abbreviations; r, residual.

N containing leaf displayed 28% increase in *Cr* over that feeding 4 week old leaf (Taylor and Bardner 1968). A comparison of Pe_2 of *D. chrysippus* feeding on senescent leaf (VI instar: 13·6%) with that of *E. mollifera* (VI instar: 28·7%) suggests that *E. mollifera* compensates the poor nutritional quality of the leaf by maintaining their Pe_2 at a level far higher than that of *D. chrysippus*. The decrease in the *Cr* of larvae feeding on senescent leaf points out the role of water and N contents of the food in eliciting positive feeding responses and regulation of food intake as in several other insects (Bernays and Simpson 1982). The finding that the *Cr* of *S. litura* feeding N and water rich flower (female: 8·239 kJ/g/d) is more than that of the larva feeding tender leaf (female: 5·519 kJ/g/d) substantiates the above conclusion. Higher nutritional quality of *B. oleracea* flower enabled *S. litura* not only to utilize the flower more efficiently but also to allocate more energy to egg production. The data obtained on ingestion of tender and senescent leaf or flower by the pests confirm the conclusion of Schoonhoven (1981) that plant chemicals like sugar, protein and water contents are responsible for feeding responses and regulation of food intake. The poor assimilation efficiency of the larvae in the senescent leaf schedule was due to low N and water and the presence of a greater proportion of indigestible matter like fibre. It is interesting to note that Pandian and Marian (1985) have obtained a significant positive correlation between N content of food and *Ase* of lepidopterous insects.

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Effect of diethylstilbestrol on growth and food conversion of common carp, *Cyprinus carpio* (Linn.)

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Abstract. Diethylstilbestrol, an estrogen, was administered through diet to fingerlings of common carp, *Cyprinus carpio* at concentrations of 0, 1, 3 and 5 ppm over a period of 98 days and its effects on growth and food conversion efficiency were evaluated. The experiment was conducted in 12 cement cisterns of 25 m^2 area each. Fishes were fed daily at the rate of 5% of body weight. Diethylstilbestrol incorporated diets induced faster growth and better food conversion efficiency as compared to the control. Among the dosages tried, 3 ppm treatment yielded the best growth. The faster growth in diethylstilbestrol treated groups was significantly different ($P < 0{\cdot}05$) from that of the control.

Keywords. Diethylstilbestrol; *Cyprinus carpio*; growth.

1. Introduction

Hormones as feed additives are finding increased application in fish culture as a means of inducing fish growth, thereby bringing down the cost of fish production. A variety of hormones, both natural and synthetic, have been evaluated for their anabolic properties in fish. Of these, androgens have given more consistent results than estrogens, which have yielded contradictory growth responses in fish (Donaldson *et al* 1979). Diethylstilbestrol (DES), a synthetic estrogen, yielded negative growth response in channel catfish (Bulkley 1972), rainbow trout (Ghittino 1970; Matty and Cheema 1978) and coho parr (Fagerlund and McBride 1975). However, investigations on plaice, *Pleuronectes platessa* (Cowey *et al* 1973), *Channa striatus* (Nirmala and Pandian 1983) and carps (Reddy *et al* 1987; Basavaraja N, Nandeesha M C and Varghese T J, unpublished results) indicated its effectiveness as a growth promoter. Considering the relatively few trials conducted employing estrogens and also the encouraging results obtained in some of the earlier experiments, the present study was taken up to evaluate the growth promoting ability of DES in the common carp, *Cyprinus carpio.*

2. Materials and methods

The experiment was conducted in 12 similar cement cisterns of 25 m^2 ($5 \times 5 \times 1$ m) area each. The cisterns were cleaned thoroughly, dried and filled with freshwater to a depth of 65 ± 5 cm.

Eight fingerlings of common carp having an average size of 1·6 g, were stocked in each cement cistern. The water in the cisterns was periodically replenished to avoid deterioration in water quality and development of plankton. The experiment was conducted in triplicate for a period of 98 days.

Standard pelleted fish-feed developed at the College of Fisheries, Mangalore

(Varghese *et al* 1976) was used as the medium of hormone administration. Details of the composition of the experimental diets are given in table 1. The required quantities of DES were weighed accurately and dissolved in 95% ethanol. The hormone dissolved ethanol was sprayed on cooked and cooled feed mixture. The DES was incorporated in the diet at dosage levels of 1, 3 and 5 mg/kg. After thorough mixing, the dough was pelletized and dried at a temperature below 50°C to a moisture content of less than 10%. Control diet was also prepared in the same manner using only ethanol. The diet used had 30·48% protein, 5·26% fat, 11·37% fibre, 15·78% ash, 28·50% N-free extract and 13144 joules/g calorific content.

The experimental fish were sampled at fortnightly intervals to assess the growth. After every sampling, the quantum of feed to be given was readjusted in accordance with the fish weight. Water quality parameters of the experimental cisterns, such as pH, temperature, dissolved oxygen, free carbon dioxide and total alkalinity, were monitored at fortnightly intervals. On termination of the experiment, all the surviving fishes were collected by dewatering the cisterns and their individual weights recorded. Growth data were analysed by employing '*t*' test to test for significant difference between final average weights of experimental groups and the control. The percentage specific growth (SGR) and food conversion efficiency (FCE) were calculated using the following formulae.

$$\% \text{ SGR} = \frac{\log W_2 - \log W_1}{T_2 - T_1} \times 100.$$

Where W_2 = weight at time T_2; W_1 = weight at time T_1.

$$\text{FCE} = \frac{\text{Weight gain (g)}}{\text{Feed intake (g)}} \times 100.$$

3. Results

The survival of the experimental fish was 75% in control, 1 and 5 ppm treatments, whereas it was 83·3% in 3 ppm treatment. Data on the growth of fish in different treatments are given in table 2 and figure 1. From this data it is evident that the fish administered DES grew significantly faster than the controls ($P < 0{\cdot}05$). Average net gain in weight was the highest in 3 ppm treatment (64·60 g) followed by 5 ppm (54·90 g), 1 ppm (50·9 g) and control (45·20 g), the corresponding average daily increments in weight being 0·66, 0·56, 0·52 and 0·46 g. Specific growth rate values of

Table 1. Percentage composition of the experimental diets.

Ingredients	Diets			
	Control	1 ppm	3 ppm	5 ppm
Rice bran	40	40	40	40
Fish meal	25	25	25	25
Oil cake	24	24	24	24
Tapioca	10	10	10	10
Mineral mix (Nuvimin forte)*	1	1	1	1
DES (mg/kg)	—	1	3	5

*Sarabhai Chemicals, Baroda.

Table 2. Effect of DES supplementation on growth of *C. carpio*.

Parameter	Treatments			
	Control	1 ppm	3 ppm	5 ppm
Average initial weight (g)	1·60	1·60	1·60	1·60
Average final weight (g)	46·80 ± 15·27	52·50 ± 7·10*	66·20 ± 26·04*	56·50 ± 9·53*
Net weight gain (g)	45·20 ± 15·27	50·90 ± 7·10	64·60 ± 26·04	54·90 ± 9·53
Weight gain over control (%)	—	12·61	42·92	21·46
Food conversion efficiency (%)	43·09 ± 3·35	48·07 ± 0·92	51·21 ± 5·28	48·84 ± 8·02
Specific growth rate (%)	3·42 ± 0·34	3·56 ± 0·14	3·72 ± 0·39	3·60 ± 0·19

*Significant at 5% level.

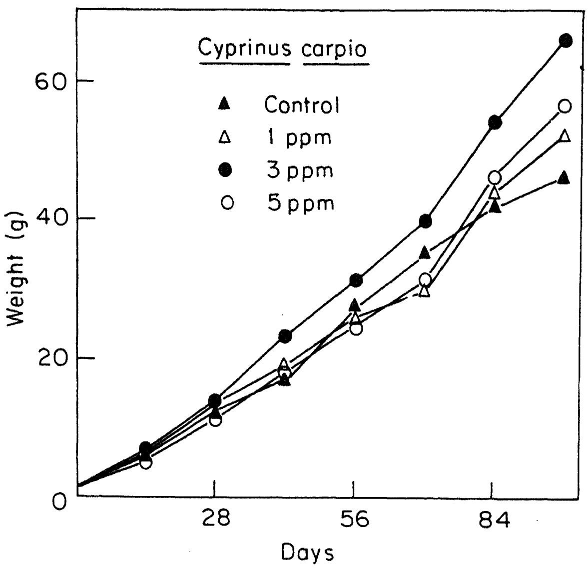

Figure 1. Average weight attained by common carp in different treatments.

3·42, 3·56, 3·72 and 3·60 were recorded for fishes under control, 1, 3 and 5 ppm treatments respectively. Better FCE was recorded in the steroid fed groups (table 2), the values being 43·09, 48·07, 51·21 and 48·84% in control, 1, 3 and 5 ppm treatments respectively.

4. Discussion

The results of the study clearly show that the dietary incorporation of DES significantly enhances growth ($P < 0·05$) in common carp. The percentage weight gains over control in the different treatments were 12·61, 42·92 and 21·46 in 1, 3 and 5 ppm treatments respectively. On the basis of the net percentage weight gain over the control, it appears that DES at 3 ppm level induces the best growth. Values of specific growth rate obtained also support this observation. While treating common carp with DES at concentrations of 5 and 10 ppm it was observed that the former

dosage induced growth and the latter resulted in growth depression (unpublished results). Hence, at higher levels DES appears to act catabolically resulting in growth depression. Growth promotion on DES administration was reported by Cowey *et al* (1973) and Nirmala and Pandian (1983). On the other hand, Bulkley (1972), Chittino (1970), Fagerlund and McBride (1975) and Matty and Cheema (1978) observed depression of fish growth on treatment with DES. However, trials conducted in carps have given encouraging results (Reddy *et al* 1987; Basavaraja N, Nandeesha M C and Varghese T J, unpublished results). The results of the present study also support the anabolic properties of DES on carps and indicates that the optimum dosage of DES required for growth promotion in common carp is around 3 ppm.

Increase in FCE was noticed in the steroid treated groups. The percentage increase in FCE were 11·56, 18·84 and 12·95 in case of 1, 3 and 5 ppm treatments respectively. The results of the present study have great economic implications in intensive carp culture since supplementary feeding alone accounts for about 50% of cost of fish production. The percentage increase in food conversion efficiency in 3 ppm DES treatment reveals that the cost of production can be brought down considerably by incorporation of DES in the diet at this level. Due to incorporation of DES at 3 ppm level, the cost of feed will increase only by about 20 paise per kg at the present price of the steroid. Though steroid hormones are reported to be metabolised and excreted from the body quickly, it is necessary to reconfirm this before advocating the use of DES as a feed additive in carp culture.

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Seasonal changes in the timing of hopping and feeding activities of a tropical bird (*Estrilda amandava*) under natural photoperiod

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Abstract. The timings of onset and end of hopping and feeding activities of the bird *Estrilda amandava*, were studied under natural light-dark cycles and they are found to keep pace with the timings of sunrise and sunset. The onsets of hopping and feeding occurred at different timings of twilight and they vary over the seasons. The phase angle difference (ψ) of hopping always shows a considerable phase lead relative to feeding which also varies over the seasons and thus suggests that there may be two endogenous oscillators, one responsible for hopping and the other for feeding.

Keywords. *Estrilda amandava*; hopping; feeding; photoperiod; phase angle difference.

1. Introduction

Many bodily functions of organisms oscillate rhythmically and keep pace with the geophysical temporal order (Bünning 1973, 1982). In higher vertebrates, the main entraining agent or 'zeitgeber' is the natural light-dark cycle (Aschoff *et al* 1982). In animals, locomotor activity is best suited to demonstrate the seasonal changes in temporal relationship between activity cycles and environmental cycles (Daan and Aschoff 1975). The timings of onset and end of activity can be easily used as reference points for their rhythms (Chandrashekaran *et al* 1983).

In recent years, many investigators have made systematic field studies on seasonal trends in the timings of activity in birds and mammals (Daan and Aschoff 1975; Kenagy 1976; Pohl and West 1976). Several studies specifically illustrate the temporal disparity between feeding and activity patterns under various photoperiods (Gänshirt *et al* 1984). In the present study, we made observations on hopping and feeding activities of the tropical bird red-headed munia, (*Estrilda amandava*) under natural photoperiod and we present evidence that these birds exhibit marked changes in the temporal relationships between hopping and feeding activities in relation to the environmental parameters.

2. Materials and methods

Red-headed munia birds (*E. amandava*) were caught (n = 50–75) near the University campus and kept in the outdoor aviary (5·8 × 2·4 × 2·4 m). They were fed with millets and grains (*ad lib*) . Visual observations on the timings of onset and end of hopping and feeding were made for 10 months (from July–April). The time of hopping and feeding of the first bird was considered as the index of onset of activity

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and onset of feeding of the flock respectively. Similarly, the time at which the last bird roosted was considered as the end of activity of the flock and the feeding time of the last bird was considered as the end of feeding. Sunrise and sunset times were obtained from the tables of the Indian Ephemeris Nautical Almanac published by the Director of Observatories, Calcutta and were adjusted for longitude, latitude and Indian Standard Time (IST). Seasonal variations of timings of onset and end of hopping and feeding were used to calculate the phase angle difference (ψ) and activity time following the method of Daan and Aschoff (1975) and Kenagy (1976).

The phase angle differences (ψ_o, ψ_e, ψ_m) of hopping and feeding are calculated as follows:

ψ onset (ψ_o) = Time difference between sunrise and onset of activity.
ψ end (ψ_e) = Time difference between sunset and end of activity.
ψ midpoint (ψ_m) = $\frac{1}{2}(\psi_{onset} + \psi_{end})$.

3. Results

The duration of activity period was measured as the time elapsed between the first hopping and the last roosting of the birds. Similarly, the duration of feeding period of the flock was measured as the time elapsed between the onset and end of feeding activities of the flock. The timings of onset and end of activity and feeding systematically changed and paralleled the timings of sunrise and sunset respectively (figure 1). The duration of activity was always longer than the photoperiods and the duration of feeding was always shorter than the photoperiods. The duration of activity and feeding (figure 2) are linearly correlated with the duration of daylength ($r = 0{\cdot}85$ for activity period and $r = 0{\cdot}9$ for feeding period).

The temporal relationship between the biological and environmental cycles can be expressed by the phase angle difference (ψ) of the onset (ψ_o) and end (ψ_e) of activity (Daan and Aschoff 1975). The theory of oscillation suggests that day active animals have largest ψ_o and smallest ψ_e during shorter photoperiods; smallest ψ_o and largest ψ_e during longer photoperiods. The birds have their largest and smallest ψ_o of hopping and feeding during shorter and longer photoperiods respectively. The largest ψ_e of hopping and smallest ψ_e of hopping and feeding occurred during longer and shorter photoperiods respectively concurring with the theory of oscillation. However the largest ψ_e of feeding did not occur during longer photoperiods (March–July) but during January (figure 3).

According to Aschoff (1965, 1969) a third measure of phase angle difference between the activity rhythm and the entraining light-dark cycles is ψ-midpoint (ψ_m) i.e. the time interval between the midpoint of activity and midpoint of daytime. The seasonal course of ψ_m of activity showed relatively lesser variations compared to ψ_o and ψ_e. The birds have less negative or more positive ψ_m values during longer photoperiods and more negative or less positive ψ_m values during shorter photoperiods. Similar is the case with the ψ_m of feeding activity (figure 3).

4. Discussion

The hypothesis that the daily activity of an animal depends upon an endogenous timer and re-setting by environmental cues is not peculiar to birds and our data

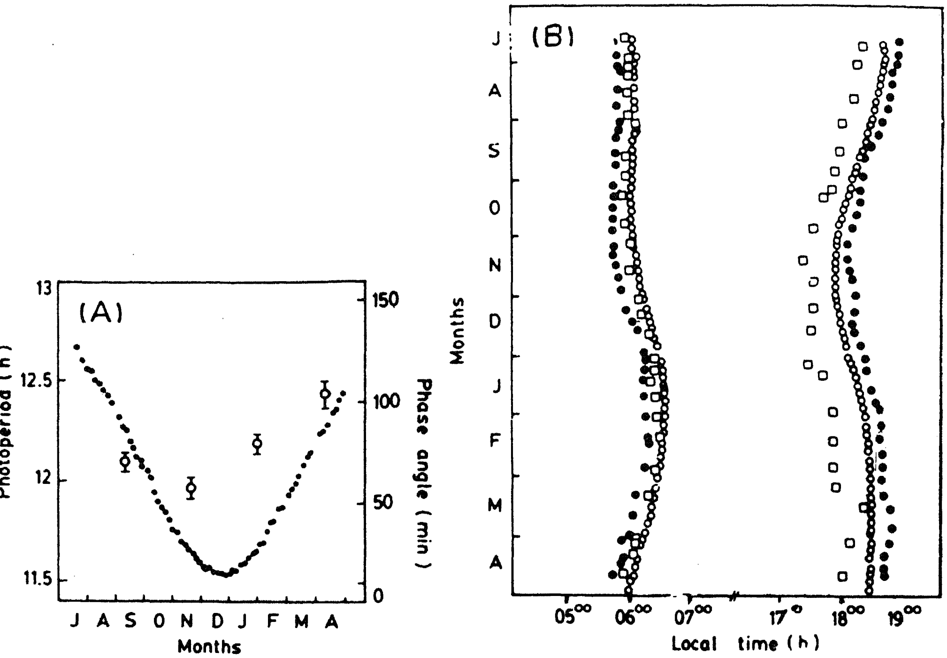

Figure 1. **A.** Photoperiod over the seasons. The time difference (phase angle) between hopping and feeding onsets over the seasons (Mean ± SD) are plotted. Note that during longer photoperiods the difference is larger and during shorter photoperiods the difference is lesser. **B.** Timings of onset and end of hopping and feeding under natural photoperiod. (○), Timings of sunrise and sunset; (●), onset and end of hopping; (□), onset and end of feeding.

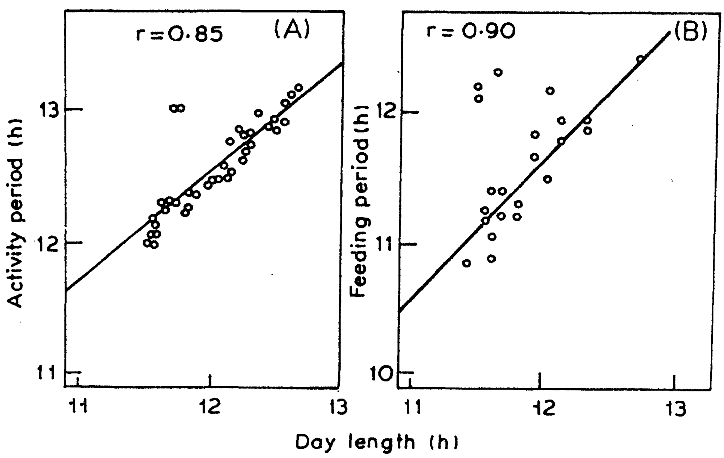

Figure 2. Linear regressions of duration of hopping and feeding with daylength.

merely broaden the comparative base of a well-grounded theory formulated by several authors (Bruce 1960; Pittendrigh 1960; Aschoff 1963). The comparison of activity time (α) as a linear function of daylength (figure 2) shows that α follows the seasonal variation rather closely (Kenagy 1976; Pohl and West 1976; Erkert 1978;

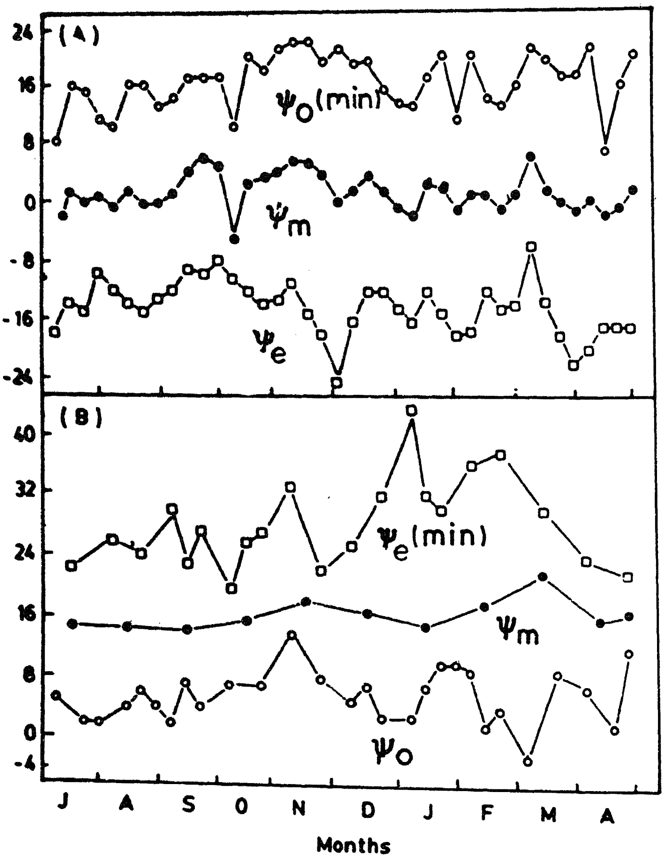

Figure 3. Seasonal changes in the phase angle differences (min) (ψ_o, ψ_e, ψ_m) of hopping (A) and feeding (B) activities of the bird flock over the seasons.

Georgii 1981). In temperate regions α follows the seasonal variations of daylength only to a limited extent. For example, in day-active birds an S-curved relationship was reported by Daan and Aschoff (1975). The activity time (duration of activity) on days shorter than about 5 h and days longer than 19 h is independent of daylength. In Madurai (9°59′N 78° 10′ E) the daylength does not vary much (0·26 min/day) and hence an S-shaped relationship could not be obtained.

The feeding time also shows a linear variation with daylength (figure 2). The time difference (phase angle) between ψ_o of hopping and ψ_o of feeding is lesser (59 min) during shorter daylengths and larger (102 min) during longer daylengths. The seasonal variations of ψ_o and ψ_e of hopping and feeding tend to exhibit an approximate mirror image (figure 3). This phenomenon of mirror imaging might have arisen through the events of light intensity variations during onset and end of activity over the seasons (Daan and Aschoff 1975).

Gänshirt *et al* (1984) reported that the hopping activity always had a phase lead relative to feeding in starlings (*Sturnus vulgaris*). In our birds too, the onset of hopping always had a phase lead over onset of feeding and the time difference is lesser (59 min) during shorter daylengths and larger (102 min) during longer

daylengths similar to *S. vulgaris* (figure 1) (Gänshirt *et al* 1984). Such temporal disparity between activity and feeding might suggest that there may however, be two endogenous oscillators, one responsible for hopping and another one for feeding as claimed by Gänshirt *et al* (1984). But it is desirable to conduct experiments under controlled laboratory conditions (under dim LL or DD) to find out whether hopping and feeding are controlled by two separate endogenous oscillators in this tropical bird.

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'heromonal and hormonal control of reproduction in the freshwater rawn, *Macrobrachium kistnensis*

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Abstract. In the freshwater prawn *Macrobrachium kistnensis* reared with males, the ovarian development occurred normally, whereas it was delayed in the absence of males. Testis and vas deferens extracts when added to the water in which females are being reared brought about normal development of ovary in comparison to those which were reared in boiled water or 'female water' alone. Therefore, mature males appear to be producing a pheromone which is necessary for the ovarian development of the female and the source of this might be vas deferens or testis. On the other hand, the central nervous tissue extracts stimulated vitellogenesis in oocytes. In particular, the brain extracts were more effective than those of thoracic ganglion in inducing this response. Thus, in the present study ovarian maturation and vitellogenesis appear to be under the pheromonal and hormonal controls.

Keywords. Pheromone; hormone; reproduction; *Macrobrachium kistnensis*.

. Introduction

\n endocrine mechanism for ovarian development in the prawn, *Leander serratus* vas first reported by Panouse (1943, 1944, 1946) who found that eyestalk ablation nduced rapid ovarian development in the nonbreeding season; selective surgical emoval of the sinus gland from the eyestalk accelerated egg laying and implants of inus glands into eyestalkless prawns prevented the otherwise anticipated ovarian levelopment. Similar results have been reported for the crayfish, *Cambarus immunis*; he crabs, *Uca pugilator* (Brown and Jones 1947, 1949) and *Paratelphusa hydrodromous* (Anilkumar and Adiyodi 1980, 1985) and the shrimp, *Paratya compressa* Takewaki and Yamamoto 1950).

The presence of an ovary stimulating factor in the brain and thoracic ganglion ias been shown in several brachyuran species. These findings led to the current elief that, in crustaceans ovary growth is under dual hormonal control (Adiyodi ınd Adiyodi 1970; Adiyodi and Subramoniam 1983; Adiyodi 1985).

Ovarian development in the freshwater shrimp, *P. compressa* was delayed in the ibsence of the male. On the other hand if they are reared along with the males ıormal ovarian development occurred. Similar results were obtained when the emales were reared with water containing the extracts of the testis or vas deferens Takayanagi *et al* 1986).

These results show that mature male shrimps secrete an ovary stimulating pheromone. The effectiveness of the central nervous system and the pheromones produced by the male in stimulating ovarian growth of the freshwater prawn, *Macrobrachium kistnensis* has been presented in this paper.

2. Materials and methods

Animals used in these studies were kept in aquaria under a light cycle of L:D

14:10 at 25°C. Animals were fed on wheat floor and water was changed every 4 days; they were kept under these conditions for a week prior to use.

The ovaries were fixed with Bouin's solution, dehydrated by alcohol, and embedded in paraffin wax. Serial sections of 8 μm were stained with Harri's haematoxylin eosin.

Brain, thoracic ganglion and muscle removed from mature females was homogenized in glass distilled water for 15 min at 0°C. Each extract was centrifuged at 2000 rpm for 10 min and the supernatant (1 organ/animal) was added to 2 l of water.

The testis and vas deferens removed from mature males was homogenized as described above.

3. Results

When prawns were reared in the company of males, the ovary developed which is evident by the colour (yellowish to dark green), increased number of vitellogenic oocytes and increase in the diameter of the oocytes. When the prawns were reared in isolation, the ovarian growth was significantly low in comparison to those reared along with males (table 1).

When prawns were reared in the water in which males and females lived together, the ovary of the females showed greater development (colour changes from white to dark green, vitellogenic oocytes, and oocyte diameter increases) in comparison to those reared in isolated 'female water' and 'boiled water' (table 1).

Testis or vas deferens water significantly increased the ratio between previtellogenic, vitellogenic oocytes and oocyte diameter in comparison to the ovary of those reared in muscle water (table 1).

Oocytes of the ovary reared in thoracic ganglia or brain extracts were larger than those of controls (table 1), the brain extract being more effective than the extract of thoracic ganglion. Mean oocyte diameter was more than 320 μm with the ganglionic extracts compared with about 252 μm for the controls.

4. Discussion

The ovary of the freshwater prawn, *M. kistnensis* matured at a normal rate when reared along with the males, whereas in isolation it took a longer time to mature. Takayanagi *et al* (1986) reported similar results in the freshwater shrimp, *P. compressa*. Ovarian development also occurred normally in the prawns reared with water containing extracts of the testis or the vas deferens. Therefore, mature male prawns are shown to secrete an ovary stimulating pheromone and the sources may be some male organs such as the testis and the vas deferens. Similar findings were reported in the freshwater shrimp, *P. compressa* (Takayanagi *et al* 1986). On the other hand, the extracts of the central nervous system stimulated vitellogenesis in oocytes. In particular, the brain extracts were more effective than those of thoracic ganglion in inducing this response. These observations support the cytological studies of Matsumoto (1962) and Erribabu *et al* (1980) that the thoracic ganglion has an ovarian stimulating hormone. The results of Eastman-Reks and Fingerman (1984) on the crab, *U. pugilator* and Takayanagi *et al* (1986) on the shrimp, *P. compressa* support our findings, where an ovary stimulating hormone is

Table 1. Effect of various rearing conditions on ovarian development of *M. kistnensis* (rearing was done for 45 days).

Rearing conditions	Colour of ovary		Ratio of previt : Vitell. oocytes		Oocyte diameter $\mu m \pm SD$	
	Immature	Maturing	Immature	Maturing	Immature	Maturing
Rearing with male "male water"	Creamy	Dark green	12:5	0:9	$142·4 \pm 3·6$	$287·3 \pm 6·5$
Isolated rearing "female water"	Whitish	Faint green	20:3	7:8	$108·3 \pm 5·6$	$201·6 \pm 5·4$
Rearing in "male water"	Green	Dark green	6:10	0:5	$147·0 \pm 6·8$	$328·4 \pm 6·7$
Rearing in "female water"	Whitish	Faint green	20:1	7:8	$100·8 \pm 5·5$	$192·8 \pm 3·7$
Boiled "male water"	White	Creamy	20:2	7:7	$102·6 \pm 4·7$	$196·6 \pm 5·1$
Boiled "female water"	White	Creamy	19:2	6:9	$105·6 \pm 4·3$	$210·6 \pm 3·8$
Rearing in muscle extract	Creamy	Green	15:3	2:9	$131·2 \pm 3·2$	$252·7 \pm 5·2$
Rearing in testes-extract	Creamy	Green	13:5	2:9	$141·6 \pm 4·1$	$260·5 \pm 4·2$
Rearing in vas deferens extract	Creamy	Green	12:6	1:9	$150·2 \pm 4·5$	$263·1 \pm 4·3$
Rearing in thoracic ganglion extract	Light green	Green	9:6	0:8	$158·6 \pm 5·3$	$298·4 \pm 4·7$
Rearing in brain extract	Light green	Dark green	6:7	0:6	$168·6 \pm 5·7$	$320·2 \pm 5·9$

shown to be present in the central nervous system and to act directly on ovarian vitellogenesis. The results of the present study indicate that the ovarian maturation, vitellogenesis in the freshwater prawn, *M. kistnensis* is under the control of pheromones produced by the male and hormones produced by the females.

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Consumption and utilization of food in different instars of muga silkworm *Antheraea assama* Westwood

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Abstract. An investigation was carried out to find out the nutritional parameters viz. consumption, assimilation and tissue growth etc. of muga silkworm *Antheraea assama* Westwood (Lepidoptera: Saturniidae) by rearing it indoor on som twigs. During 32 days of its larval life, the total consumption of an individual was 33·925 g of which 21·295 g were assimilated by the insect. About 80·1% of the total consumption took place in the fifth instar alone. The weight of one full grown larva was computed at 13·9304 g. The assimilation and tissue growth were found positively correlated with the consumption as well as with the age of the larva. The approximate digestibility was negatively correlated to the amount of food consumed. Efficiency of conversion of ingested and digested food increased in the first 4 instars and declined in the fifth instar.

Keywords. *Antheraea assama*; consumption; approximate digestibility; conversion of ingested food; conversion of digested food.

1. Introduction

Study of nutritional parameters of sericigenous insects is considered to be an important field of work for better management and development of the sericulture industry apart from its physiological importance. Considerable literature is available in this field and special mention can be made of the works of Kapil (1963), Poonia (1978), Reddy and Alfred (1979), Joshi (1984, 1985) and Pant *et al* (1986) on *Philosamia ricini*, Horie and Watanabe (1983) on *Bombyx mori* and Yadava *et al* (1983) on *Antheraea proylei*. Works of Evans (1939) and Waldbauer (1964, 1968) are also noteworthy.

In this study, we tried to evaluate certain nutritional parameters such as consumption, assimilation and tissue growth and their relationship to the efficiency to convert the ingested as well as digested food to body substances during the entire larval life of muga silkworm *Antheraea assama*, an insect of immense sericultural importance, which produces the lustrous golden muga silk.

2. Materials and Methods

The present study of nutritional factors of muga silkworm *A. assama* was based on som plant (*Machilus bombycina* King), one of the two primary food plants of the insect. Rearing of the worms from first to fifth instar was done under indoor conditions on twigs of som plants kept in bottles containing water. A total of 150 larvae were transferred in 3 replications of 50 larvae each. In order to determine the loss of weight due to desiccation, a control batch of twigs with identical numbers of

*Since deceased.

replications were also maintained. Further, a reserved batch of worms were maintained in identical rearing conditions so as to replace the dead or, weak larvae whenever required. The twigs were changed daily and the weight gain by the larvae, loss of weight of the twigs due to desiccation/consumption and the weight of the egested matters were recorded. The gravimetric method of Waldbauer (1968) and the IPB formula of Petrusewicz and MacFayden (1970) were used for estimation of daily food consumption and calculation of food energy assimilation and respiration. The method of Evans (1939) was used for conversion of assimilation and the gain in weight data to coefficient of utilization and growth.

Coefficient of utilization or approximate digestibility (AD) $= \dfrac{\text{Assimilation}}{\text{Consumption}} \times 100.$

Efficiency of conversion of ingested (ECI) food to body substances $= \dfrac{\text{Tissue growth}}{\text{Consumption}} \times 100.$

Efficiency of conversion of digested (ECD) food to body substances $= \dfrac{\text{Tissue growth}}{\text{Assimilation}} \times 100,$

where, assimilation = weight of food ingested − weight of egested matters.

3. Results and discussion

Instarwise consumption of leaves (in dry gram weight basis) by an individual larva is presented in table 1. The total consumption of a larva is computed to be 33·925 g. There was a steady increase in consumption rate up to fourth instar stage, while the increase was quite steep in the fifth instar being 80·1% of the total consumption (figure 1). Corresponding to consumption, a similar pattern of increase was reflected in the rate of assimilation and tissue growth (figure 2). A bulk of 74·1% of the total assimilation and 75·9% of the total tissue growth occurred during the fifth instar stage alone. Though 33·925 g food is consumed by a larva during its entire larval life, the total assimilation is only 21·295 g, which amounts to 62·8% of the total consumption. When the results of the increase in body weight or tissue growth is computed, a direct correlation is observed with that of the age of the larva. Soon after hatching, the average larval weight is $0{\cdot}0059 \pm 0{\cdot}00014$ g. The weight of one full grown larva before spinning attains $13{\cdot}9304 \pm 0{\cdot}322$ g, which is about 2360 times of a newly hatched larva. The present findings on consumption, assimilation and tissue growth follow a pattern found in a number of Lepidopteran insects like *Philosamia ricini* (Reddy and Alfred 1979; Poonia 1978), *Pieris brassicae* (Yadava *et al* 1979; Evans 1939) and *Antheraea proylei* (Yadava *et al* 1983; Rana *et al* 1987).

Digestibility, ECI and ECD food to body substances are 3 primary parameters of nutrition physiology and are presented in table 2. The AD was found to be negatively correlated to the amount of food consumed. The highest AD (93·51%) was observed in first instar and the lowest (58·07%) in the fifth instar. Similar results were obtained with *P. ricini* (Kapil 1963; Poonia 1978), *A. proylei* (Yadava *et al* 1983; Rana *et al* 1987), *P. brassicae* (Yadava *et al* 1979) and certain other Lepidopteran insects (Waldbauer 1968). Selective feeding of tender leaves by the early instar larvae as suggested by Rana *et al* (1987) might be the cause of decline of AD in the late instars.

Table 1. Average consumption, egestion, assimilation, tissue growth and respiration in different instars of *A. assama* fed on som leaves (in dry gram weight basis).

Instar	Duration of instar (days)	Consumption	Egestion	Assimilation	Tissue growth	Respiration
I	5	0·169 ± 0·003 (0·5)	0·011 ± 0·001	0·158 ± 0·003 (0·7)	0·0261 ± 0·0015 (0·2)	0·1323 ± 0·0013
II	5	0·475 ± 0·010 (1·4)	0·051 ± 0·002	0·423 ± 0·008 (2·0)	0·1352 ± 0·0067 (1·0)	0·2882 ± 0·0049
III	6	1·480 ± 0·026 (4·4)	0·215 ± 0·004	1·265 ± 0·023 (6·0)	0·5998 ± 0·0039 (4·3)	0·6652 ± 0·0272
IV	6	4·617 ± 0·180 (13·6)	0·955 ± 0·014	3·662 ± 0·167 (17·2)	2·5942 ± 0·0406 (18·6)	1·0681 ± 0·1730
V	10	27·184 ± 0·299 (80·1)	11·397 ± 0·035	15·787 ± 0·268 (74·1)	10·5693 ± 0·3046 (75·9)	5·2180 ± 0·0366

Instarwise per cent of consumption, assimilation and tissue growth are given in parentheses.

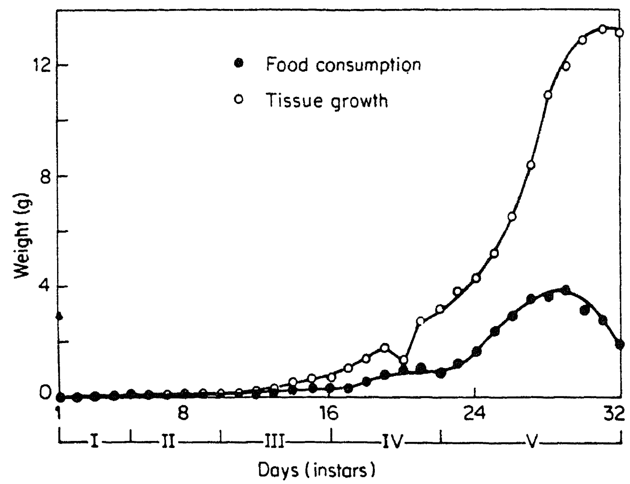

Figure 1. Daily food consumption and larval weight in *A. assama*.

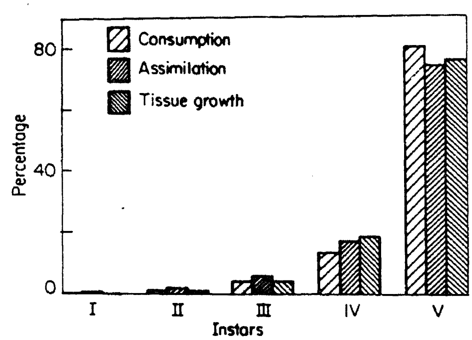

Figure 2. Instarwise per cent of consumption, assimilation and tissue growth in *A. assama*.

Table 2. Mean efficiency of food utilization by the larvae of *A. assama* fed on som leaves.

Instar	Approximate digestibility (%)	Efficiency of conversion of ingested food (%)	Efficiency of conversion of digested food (%)
I	93·51 ± 0·36	15·38 ± 0·64	16·45 ± 0·72
II	89·19 ± 0·21	28·46 ± 1·03	31·92 ± 1·19
III	85·47 ± 0·13	40·54 ± 0·99	47·44 ± 1·19
IV	79·30 ± 0·53	56·28 ± 2·42	70·99 ± 3·52
V	58·07 ± 0·35	38·87 ± 0·70	66·93 ± 0·80

As regards to ECD and ECI the values increased from first to fourth instar and declined thereafter in the fifth stage (table 2). A number of workers supported this trend of rise and fall in ECD and ECI in different insects (Bailey 1976; Yadava *et al* 1979; Rana *et al* 1987). Mukherji and Guppy (1970) and Latheef and Harcourt (1972) also supported this trend of ECD in different insects. Contrary to the above pattern, Vats and Kaushal (1982) reported gradual rise of ECD from first to the fifth instar in *P. brassicae*. The mean value of ECD and ECI are calculated to be 47

and 36% respectively in *A. assama*, while ECD and ECI of *A. proylei* and *B. mori* were reported to be 34 and 23% (Rana *et al* 1987) and 52–53 and 22–23% (Horie and Watanabe 1983) respectively. Bailey (1976) established a correlation of variation in ECI within and between the instars in respect of food plants indicating involvement of nutritional value and digestibility of different food plants.

From the above findings it is observed that both ECD and ECI decline in the fifth instar, while maximum tissue growth takes place in the same stage. Perhaps, the longer larval life and maximum consumption of food (80·1% of the total consumption) during the instar is responsible for the high rate of tissue growth inspite of the decline in ECD and ECI. The fall of ECD and ECI in the fifth instar may be due to the fact that the higher amount of digested or ingested food is metabolised for maintenance of the body and lesser amount is used for tissue growth.

The present findings on consumption and utilization of food will form an index to identify the effective strains among the primary food plants. It can also be used as a tool for selection of suitable secondary host plant varieties for further improvement.

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Osmoregulatory ability of *Penaeus indicus* H Milne Edwards in relation to varying salinities

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Abstract. Juvenile *Penaeus indicus* osmoregulated well between 3–26‰ S for 24 and 48 h duration with isosmotic points ≈S 18‰ and ≈S 14‰ respectively. Adults also showed good osmoregulatory capability between 5–30‰ S with isosmotic points ≈S 21‰ and ≈S 17‰ for 24 and 48 h duration respectively. A duration of 48 h is essential for prawns to adjust to the new medium. Influence of various neuroendocrine centres on the osmolal concentration of haemolymph was studied. In eyestalk ablated prawns osmolal concentration decreased with time and reached a lowest after 48 h. Eyestalk ablated prawns injected with extracts of eyestalk, brain and thoracic ganglia did not show any decrease in the values with time but remained on par with the values of normal ones.

Keywords. Osmoregulatory ability; neuroendocrine control; *Penaeus indicus.*

1. Introduction

Most marine animals either actively regulate the osmolality of their body fluids or passively conform to the salinity fluctuations of the external medium (Bursey and Lane 1971). Members of the genus *Penaeus*, are distributed over a wide range of salinities and are being cultured under variety of conditions in many tropical and sub-tropical parts of the world. During their life cycle many penaeid shrimps are found to have a common migratory behaviour of returning to more saline conditions for maturation and spawning (George and Vedavyasa Rao 1968; Castille and Lawrence 1981). The juveniles of many penaeids have also been found to enter the estuaries and move to shallow brackish nursery grounds to continue their growth (Panniker 1968). Such migratory patterns would reflect the osmoregulatory capabilities of the species in question. In recent years, certain aspects of osmoregulatory capabilities of some penaeid prawns have been documented (Castille and Lawrence 1981; Dall 1981; Howe *et al* 1982; Ferraris *et al* 1986).

Evidence of neuroendocrine control in hydromineral regulation in decapod crustaceans has been proved from time to time (Bliss *et al* 1966; Charmantier *et al* 1981). The role of eyestalk hormones, brain and thoracic ganglion in the ionic regulation and water balance has also been demonstrated (Tullis and Kamemoto 1971; Heit and Fingerman 1975; Kiron and Diwan 1984a, b). Schreiner *et al* (1969) pointed out the importance of abdominal ganglion in water balance of *Homarus* sp. However, there is a paucity of information on osmolal concentration and effects of neuroendocrine factors in penaeid shrimps. The osmoregulatory behaviour of *Penaeus indicus* is not thoroughly understood. Therefore, attempts were made to compare the responses of osmolal concentrations of the haemolymph of juvenile and adult prawns over a range of salinities varying from 3–40‰ and the influence of eyestalk, brain and thoracic ganglion in the regulation of haemolymph osmolality was determined.

2. Materials and methods

Juvenile and adults of *P. indicus* were collected from the grownout ponds of the Marine Prawn Hatchery Laboratory, Narakkal, Kerala. The size range of juveniles varied between 75–85 mm TL and for adults between 140–170 mm TL. Prawns were maintained in the laboratory for 48 h. Only intermoult stage prawns were segregated for the experimental purpose. The required experimental salinities were prepared by dilution of seawater with freshwater and the salinity measured as a direct reading of the salinometer. Higher salinities were prepared by partial freezing of seawater and removal of the ice formed thereby. The range of salinities for experimental media varied from 3–40‰. Six juveniles and adult prawns were released into each of the media. Three juvenile and three adult prawns were sampled from each of the media for haemolymph extract after 24 and 48 h duration. Haemolymph sample from individual prawns was collected from the pericardial cavity using chilled 1 ml hypodermic syringe previously rinsed with a anticoagulant (10% trisodium citrate). The haemolymph was delivered into small glass vials and kept in an ice water bath until further use. From each glass vial, 50 μl of haemolymph was pipetted with the help of an automatic micropipette and immediately transferred to the osmometer cuvett. The cuvett was further transferred to osmometer (Gonotech-Osmomat-030) where the value of osmolality (freezing point depression) was directly determined. Osmolal concentration of the water of each medium was also measured simultaneously and checked with chloride determination on salinometer.

To find out the possible role of different neuroendocrine centres, in the regulation of osmolality of haemolymph, the following experimental set up was designed. Prawns in the size range of 138–148 mm were divided into 6 batches (A–F), each batch consisting of 30 animals. Each batch was further divided into 10 groups each with 3 animals. All the prawns were maintained in plastic pools having a salinity nearest to the isosmotic points (672 mOsm/kg) of the haemolymph which was determined earlier.

Bilateral eyestalk surgery was performed by using electric cauterizer for prawns belonging to all batches except A and B. The prawns of batch A treated as the initial control and batch B sham operated. The extract of different neuroendocrine organs viz. eyestalk, brain and thoracic ganglion were prepared by homogenising the tissues separately in isosaline filtered seawater and subjected them for centrifugation at 8000 g for 10 min. Supernatant of each tissue was used as the injecting material to the test animals. Eyestalk ablated prawns of batches D, E and F were injected with eyestalks, brain and thoracic ganglion extracts respectively in the ratios of 2 eyestalks/0·2 ml/prawn, 0·2 ml/brain/prawn and 0·2 ml/thoracic ganglion/prawn. Ablated prawns of batch C were injected with 0·2 ml/prawn isosaline filtered seawater. After initiation of the experiment first haemolymph sample was collected immediately from the first group of prawns of all the batches. The next sampling was after 1 h and then at intervals of 2, 4, 8, 12, 18, 24, 48 and 72 h. The haemolymph was then delivered into the prechilled glass vials and osmolality was determined as per the method described earlier.

3. Results and discussion

The osmolal concentrations of the haemolymph of juveniles and adult *P. indicus*

naintained in different salinities for 24 and 48 h duration are indicated in figures 1 .nd 2. Duration of 48 h was essential for prawns to adjust to the new medium and egulate normally. Both juveniles and adults showed hyperosmotic behaviour in ɔwer salinities (up to 15‰ S) and hyposmotic behaviour in higher salinities. In ıigher saline media, there was corresponding increase in the osmolal concentration ɔf haemolymph of both juveniles and adult prawns. It was evident that juvenile ɔrawns osmoregulated well in salinities 3–26‰ S for 24 and 48 h, with isosmotic ɔoints ≈S 18‰ and ≈S 14‰ respectively (figure 1). Adults also showed good ɔsmoregulatory capability between 5–30‰ with isosmotic points ≈S 21‰ and ≈S 7‰ for 24 and 48 h duration respectively (figure 2). The results of the present nvestigations indicated hyperosmotic regulation of this species at low salinities and ıyposmotic regulation at high salinities for both juvenile and adult and are in .greement with earlier reports (Williams 1960; McFarland and Lee 1963; Bursey .nd Lane 1971). The differences in the haemolymph concentrations at low and high alinities between juveniles and adults showed that while adults are better hyper-ɔsmotic regulators, juveniles are better hyposmotic regulators. Most of the penaeid pecies as reported earlier, are able to adopt extremely well to very low salinities luring their early juvenile life but this ability appears to be reduced in adults (Dall 981). Unlike the juveniles of other penaeids, the hyperosmotic behaviour of uveniles of *P. indicus* at low salinities was not pronounced in the present study. This is probably because the juveniles selected were in the late phase of their growth (size range between 75–85 mm). However, when isosmotic points are com-ɔared, juveniles showed lower isosmotic points than that of adults clearly indicating hat the animals prefer lower saline media in the early phase of their growth. In ıdult prawns the capacity to osmoregulate both at low and high salinities was ound to be extremely good. There is great diversity in osmoregulatory ability ımong penaeid species and other decapod crustaceans. Some have feeble powers of

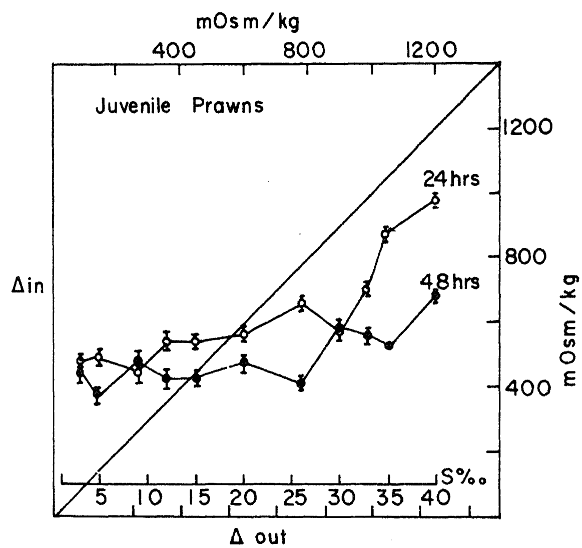

Figure 1. Osmolality of the haemolymph of juvenile *P. indicus* acclimated to different salinities.

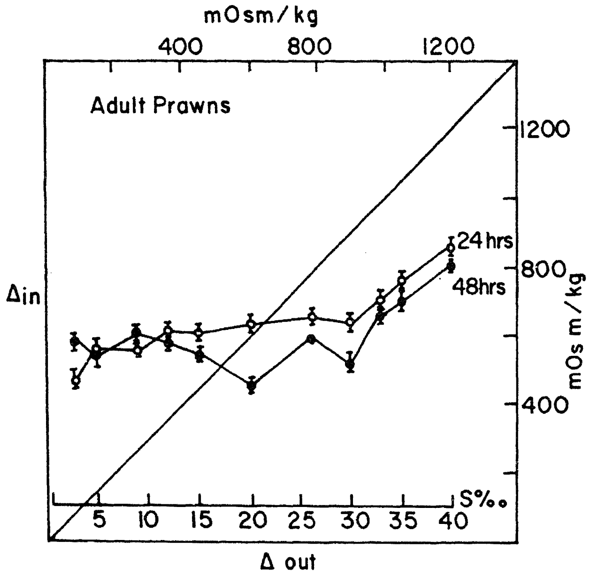

Figure 2. Osmolality of the haemolymph of adult *P. indicus* acclimated to different salinities.

osmoregulations, in some the larvae can osmoregulate but adults are osmoconformers, in others both larvae and adults have similar osmoregulatory patterns (Kalber 1970; Forskett 1977). Therefore, the osmoregulatory ability appears to be a purely adaptative feature and may change markedly during development and according to the environmental situation.

Both juvenile and adult *P. indicus* require at least 48 h for exhibiting stability in the haemolymph. Generally, when the prawns are acutely transferred to different salinities, there is a rapid change in the osmolal concentration of haemolymph and to reach a steady state equilibrium the animals requires time. Bursey and Lane (1971) have reported that for *P. duorarum* a period of about 24 h is required to establish a new steady state equilibrium for haemolymph concentration. Castille and Lawrence (1981) reported 3–4 days for *P. satiferus* to stabilize the haemolymph.

Table 1 summarises the influence of various neuroendocrine centres on the osmolal concentrations of haemolymph in *P. indicus*. Initial rise was seen in osmolal concentrations of haemolymph in eyestalk ablated prawns as also in other groups where ablation and injection of various neuroendocrine elements were conducted. In ablated prawns with isosaline seawater injection osmolal concentration decreased with the time and reached lowest after 48 h. Recouping effect could be seen only after 72 h. Ablated prawns injected with extracts of eyestalk, brain and thoracic ganglia did not show any decrease in the values with time but remained on par with the values of normal ones. Results presented in table 1 provide a firm evidence that the osmolal concentration of haemolymph in *P. indicus* is under the control of different neuroendocrine elements. Eyestalk, brain and thoracic ganglion have similar controlling factor (osmolal stimulating factor) which upon injection into the destalked prawns showed identical effects. Decreasing trend of osmolal concentration in bilaterally ablated prawns might be due to loss of osmolal controlling factor as a result of eyestalks removal. Recouping effect of

Table 1. Neuroendocrine control of osmolal concentration of haemolymph in the prawn *P. indicus* (mOsm/kg).

Hours after operation	Normal prawn	Sham operated control	Bilateral eyestalk surgery + sea water injection	Bilateral eyestalk surgery + injection of eyestalk extract	Bilateral eyestalk surgery + injection of cerebral ganglion extract	Bilateral eyestalk surgery + injection of thoracic ganglion extract
0	975 ± 3	1132 ± 6	905 ± 7	1307 ± 10	925 ± 13	1075 ± 8
1	889 ± 4	934 ± 3	895 ± 8	878 ± 2	979 ± 12	904 ± 7
2	889 ± 5	882 ± 5	846 ± 5	927 ± 10	876 ± 4	856 ± 16
4	872 ± 18	874 ± 8	836 ± 3	852 ± 4	876 ± 4	846 ± 7
8	859 ± 5	864 ± 10	801 ± 3	877 ± 3	881 ± 9	877 ± 4
12	855 ± 4	870 ± 8	826 ± 7	868 ± 2	837 ± 12	874 ± 38
18	838 ± 17	873 ± 7	823 ± 10	878 ± 33	869 ± 5	856 ± 6
24	847 ± 27	861 ± 6	780 ± 4	908 ± 25	939 ± 53	895 ± 46
48	883 ± 7	883 ± 11	708 ± 2	858 ± 6	876 ± 4	899 ± 9
72	879 ± 36	875 ± 15	820 ± 5	838 ± 5	854 ± 19	896 ± 5

Analysis of variance showed that the means of different treatments and at different time intervals differed significantly at 5% level.
Each value represents a mean of 3 determinations.
$\bar{X} \pm SD$.
Intramuscular injection: 0·2 ml/two eyestalks/prawn; 0·2 ml/brain/prawn; 0·2 ml/thoracic/prawn ganglion. Temperature of the medium = 26°C; mOsm of the medium = 672; Size range of the prawns = 138–148 mm.

retaining normal values of osmolality in the ablated prawns after 48 h could be due to the release of osmolal stimulating factor from brain and thoracic ganglia. But the response of haemolymph osmolality to various neuroendocrine elements generally depends on ionic concentration of the surrounding medium. Heit and Fingerman (1975) have reported that eyestalkless crabs did not respond to blood sodium in hyperosmotic seawater but there was a drop in the levels either in isosmotic or hyposmotic media. Similar observations were earlier made by Kamemoto *et al* (1966) while testing the extracts of eyestalk, brain and thoracic ganglia on *Procambarus clarkii* and *Metopograpsus messor*. Haemolymph chloride elevating factor has also been reported by Nagabhushanam and Jyoti (1977) in *Caridina weberi* and Venkatachari *et al* (1979) in *Barytelphusa guerini* in which removal of this factor through extract injections had elevated it to normal level. In our earlier observations we found that there was an increase in sodium and chloride ion concentration of haemolymph in eyestalkless *P. indicus* (Kiron and Diwan 1984a, b) and the present investigation indicated decrease in osmolal concentration of haemolymph in eyestalkless prawns. The reason for this difference is due to the difference in the experimental ambient medium one being hyperosmotic (Kiron and Diwan 1984a, b) and the present one isosmotic. Therefore, the osmolal controlling factor which is present in the neuroendocrine centres reacts according to the animal's need depending on the surrounding medium.

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Hydroxysteroid dehydrogenases in the ovary of larva, pupa and adult eri silkworm, *Philosamia ricini* (Hutt.) — A histochemical study

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Abstract. Histochemical analysis of the various developmental stages of *Philosamia ricini* showed the presence of Δ^5-3β-hydroxysteroid dehydrogenase, 17β-hydroxysteroid dehydrogenase, 11β-hydroxysteroid dehydrogenase and glucose-6-phosphate dehydrogenase in the lamellar epithelial cells, precursors of oocytes, nurse cells and follicle epithelial cells of the germarium of larva and pupa, oocyte-nurse cells complex, follicle epithelial cells of immature and mature follicles and intermediate cells of pupa suggesting that these tissues are able to metabolize Δ^5-3β-hydroxysteroids, 17β-hydroxysteroids and 11β-hydroxysteroids to corresponding ketosteroids.

Keywords. Eri silkworm; ovary; vitellogenesis; hydroxysteroid dehydrogenases.

1. Introduction

Steroids other than ecdysones are known to occur in the ovaries of invertebrates (Gottfried *et al* 1967; Carreau and Drosdowsky 1977; Schoenmakers and Voogt 1980). *In vivo* studies have shown that the ovary of the locust, *Schistocerca gregaria*, is capable of transforming vertebrate steroids (Dube and Lemonde 1970). Recently the presence of estradiol in the ovary of *Bombyx mori* and its role in the ovarian development have been studied (Ohnishi *et al* 1985; Ogiso and Ohnishi 1986; Ogiso *et al* 1986). In the present investigation histochemical technique has been employed to demonstrate the presence (or absence) of the enzymes involved in the metabolism of steroids in the ovaries of larva, pupa and adult *Philosamia ricini*. In addition the distribution of glucose-6-phosphate dehydrogenase (G-6-PDH), in the ovary of this silkworm has also been studied.

2. Materials and methods

The larva, pupa and adult *P. ricini* were obtained from the laboratory bred stock. For histological study the ovaries of respective stages were fixed in Bouin's fluid for 20–25 h. The tissues were processed in alcohol grades and embedded in paraffin wax and 5 μm sections were cut and stained with haematoxylin and eosin.

For histochemical studies, the larva, pupa and adult were decapitated and the ovaries were removed immediately and frozen over dry ice vapour at -50°C and sectioned in a cryostat at -20°C. The histochemical methods employed are similar to those employed for the steroid hormone producing tissues of vertebrates. For the histochemical demonstration of Δ^5-3β-hydroxysteroid dehydrogenase (HSDH), 17β-HSDH and 11β-HSDH activity, the frozen sections were incubated in the media containing different substrates (table 1), prepared according to the procedure

Table 1. Activity of HSDH in the ovary of larva, pupa and adult *P. ricini.*

Developmental stages and stages of follicles in *P. ricini*	Enzymes and the substrates*	Intensity of reaction**					
		Lamellar epithelium	Precursor cells in germarium	Nurse cells	Follicle epithelial cells	Oocyte	Intermediate cells
IV and V instar larvae 1st and 3rd day of each instar in 1st and 2nd stage follicles	Δ^5-3β-HSDH (DHA and pregnenolone), 17β-HSDH (testosterone and 17β-estradiol) and 11β-HSDH (11β-hydroxyandrostenedione	+ + +	Activity could not be ascertained***				
Pupae 1st, 3rd, 6th and 9th day, in 3rd stage follicles	Δ^5-3β-HSDH (DHA and pregnenolone), 17β-HSDH (testosterone and 17β-estradiol) and 11β-HSDH (11β-hydrodyandrostenedione)	+ + +	+ +	–	+ +	–	+ +
4th, 5th and 6th stage follicles	Δ^5-3β-HSDH (DHA and pregnenolone), 17β-HSDH (testosterone and 17β-estradiol) and 11β-HSDH (11β-hydroxyandrostenedione)	+ + +	+ +	+ +	+ +	–	+ + +
12th and 15th day, in 7th and 8th stage follicles	Δ^5-3β-HSDH (DHA and pregnenolone) 17β-HSDH (testosterone and 17β-estradiol) and 11β-HSDH (11β-hydroxyandrostenedione)	+ + +	+ +	+ + +	+ + +	+ + + (Peripheral region only)	+ + +
9th stage follicle	Δ^5-3β-HSDH (DHA pregnenolone),	+ + + + + + +	+ + + +	+ + + +	+ + + + + +	+ + + + + +	+ + + +
	17β-HSDH (testosterone and 17β-estradiol) and 11β-HSDH (11β-hydroxyandrostenedione)	+ + +	+ +	+ +	+ + +	+ + +	+ +

Adult moths, in immature follicles	Δ^5-3β-HSDH (DHA and pregnenolone), 17β-HSDH (testosterone and 17β-estradiol) and 11β-HSDH (11β-hydroxyandrostenedione)	Tissue absent	+ + +	+ + +	+ + +	+ + +	Cells absent
Adult moths, in mature follicles (stage 10 follicles)	Δ^5-3β-HSDH (DHA and pregnenolone), 17β-HSDH (testosterone and 17β-estradiol) and 11β-HSDH (11β-hydroxyandrostenedione)	Tissue absent	In mature follicles nurse cells (5) and follicle epithelial cells (6) are not found. It contains only the mature oocyte (7) surrounded by chorion.			+ + +	Cells absent
Atretic follicles	Δ^5-3β-HSDH (DHA and pregnenolone), 17β-HSDH (testosterone and 17β-estradiol) and 11β-HSDH (11β-hydroxyandrostenedione)	Immature follicles consisting of nurse cells (5), follicle epithelial cells (6) and oocyte (7) undergo atresia		+ + +	+ + +	+ + +	Cells absent

*All the chemicals are of sigma grade, obtained from Sigma Chemical Co., USA.

**Intensity of reaction is graded from minimum (+) to intense (+ + + +) activity, (−) denotes absence of reaction.

***In the IV and V instar larvae the presence of formazan granules could not be confirmed in the precursor cells, nurse cells, follicle epithelial cells and oocytes of the germarium due to their compact nature and scanty cytoplasm.

described earlier (Baillie *et al* 1966; Hurkadli *et al* 1988a). After incubation the sections were washed in distilled water fixed in 10% neutral formalin for 30 min and mounted in glycerol gelly. Parallel sections incubated in the media lacking the respective substrates served as controls. A few sections were also incubated in a medium containing dehydroepiandrosterone (DHA) and isoxozol, an inhibitor of Δ^5-3β-HSDH activity as a specific control for Δ^5-3β-HSDH activity.

3. Results and discussion

The ovary of *P. ricini* is polytrophic meroistic type. Histologically the development of the ovary leading to the formation of mature egg can be broadly divided into 4 distinct phases with 10 follicle stages (Hurkadli *et al* 1988b).

Table 1 summarises the results on histochemical reaction for HSDH in the developing and adult ovary of *P. ricini.*

During larval stage of *P. ricini* a moderate Δ^5-3β-HSDH, 17β-HSDH and 11β-HSDH activity was observed in the lamellar epithelial cells of the ovarian cap and a few formazan granules were present in the matrix cells. From fourth day of pupation a weak Δ^5-3β-HSDH, 17β-HSDH and 11β-HSDH activity was observed in the precursors of oocyte, nurse and follicle epithelial cells of stages 1 and 2 follicles in the germarium. The reaction was not noticed in the oocyte-nurse cells complex and in ovariolar sheath. A weak reaction was observed in the intermediate cell layer, in the follicle epithelial cells surrounding oocyte-nurse cells complex and in the ovariolar sheath (figure 1). Parallel consecutive frozen sections were rapidly stained with haematoxylin and eosin and observed under the microscope to identify the cells that showed formazan granules in the sections used for histochemical study.

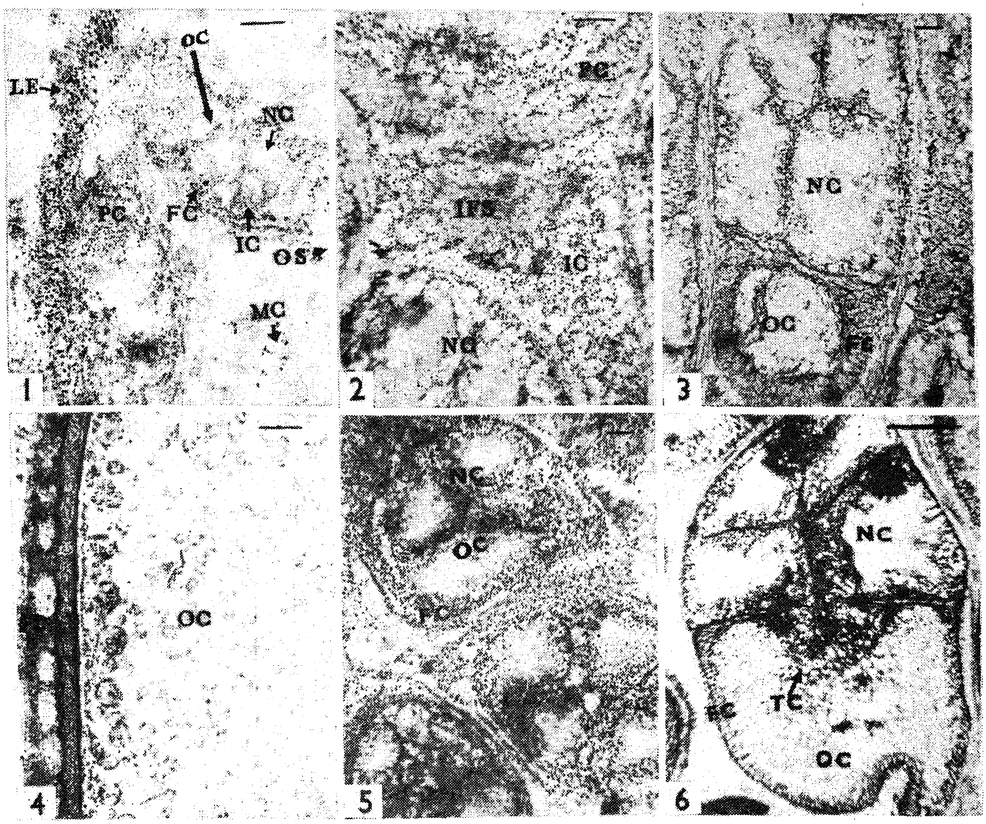
During 4th-6th follicle growth stages, a weak Δ^5-3β-HSDH, 17β-HSDH and 11β-HSDH activity was observed in the follicle epithelial cells and nurse cells. Intermediate cells showed a moderate activity for all the 3 HSDH. The activity of these enzymes were absent in the ooplasm and in the interfollicle stalk cells (figure 2).

In the 7th and 8th stage vitellogenic follicles a moderate Δ^5-3β-HSDH, 17β-HSDH and 11β-HSDH activity was observed in the follicle epithelial cells, nurse cells, intermediate cells and in the peripheral region of the ooplasm (figure 3). In the 9th vitellogenic stage follicle, the enzymes activity were spread all over in the ooplasm and slightly reduced in the nurse cells. With Δ^5-3β-HSDH activity, the substrate DHA was relatively better utilized than pregnenolone in the 9th vitellogenic stage.

In stage 10 follicles a moderate Δ^5-3β-HSDH, 17β-HSDH and 11β-HSDH activity was observed in the ooplasm and degenerating follicle epithelial cells (figure 4). The activity of these enzymes were also seen in the phagocytic follicle epithelial cells, degenerating nurse cells and ooplasm in the atretic follicles.

The intensity and distribution of 17β-HSDH activity with substrates, testosterone and 17β-estradiol were equally well utilized by the various components of the ovary during larval, pupal and adult stages.

An intense G-6-PDH activity in the lamellar epithelial cells and a moderate activity in the matrix cells of the ovarian cap of larva of *P. ricini* was observed. Some of the precursor cells in the germarium also showed a moderate G-6-PDH activity. In the 2 and 3 stage follicles, an intense G-6-PDH activity was observed in



Figures 1–6. **1.** Δ^5-3β-HSDH activity in the lameller epithelium (LE) and matrix cells (MC) of the ovarian cap, in some of the precursors cells (PC), follicle epithelial cells (FC) and intermediate cells (IC) of the stage 3 follicle in fresh frozen section of the ovary of fourth day old pupa of *P. ricini*. Note the absence of formazan granules in the oocyte (OC) and nurse cells (NC), DHA was used as the substrate. **2.** Δ^5-3β-HSDH activity in the FC, IC and NC of the fifth stage follicle in the fresh frozen section of the ovary of 9-day old pupa of *P. ricini*. Note the absence of formazan granules in the interfollicle stalk cells (IFC). Pregnenolone was used as substrate. **3.** Δ^5-3β-HSDH activity in the FC, peripheral region of the ooplasm of the OC and in the cytoplasm of the NC of the seventh stage early vitellogenic follicle in fresh frozen section of the ovary of *P. ricini*. DHA was used as substrate. **4.** Δ^5-3β-HSDH activity in the FC after the formation of chorion and in the ooplasm of the OC in the mature egg (stage 10 follicle) of the 15-day old pupa of *P. ricini*. DHA was used as the substrate. **5.** G-6-PDH activity in the FC and NC of the stage 4 follicle, in fresh frozen section of the ovary of 3-day old pupa of *P. ricini*. **6.** G-6-PDH activity in the NC, FC, trophic cord (TC) and ooplasm of the OC of stage 7 follicle in the fresh frozen section of the ovary of 12-day old pupa of *P. ricini*.
The scale line indicates 40 μm.

the follicle epithelial cells, nurse cells (figure 5). Nurse and follicle epithelial cells, interfollicle stalk cells in 5 and 6 stage follicles continued to show an intense G-6-PDH activity, while intermediate cells showed a moderate activity. In the vitellogenic follicles an intense G-6-PDH activity was observed along the tropic cord and in the peripheral region of the ooplasm (figure 6). An intense activity of this enzyme was seen in the peripheral region and a moderate activity in the

remaining part of the mature egg. The atretic follicles of *P. ricini* showed an intense G-6-PDH activity.

The *in vitro* and *in vivo* conversion of DHA to androstenedione, pregnenolone to progesterone indicate the presence of Δ^5-3β-HSDH activity in the ovaries of mollusc, *Sepia officianalis* (Carreau and Drosdowsky 1977), star fish, *Asterias rubens* (Schoenmakers and Voogt 1980) and insects, *S. gregaria* (Dube and Lemonde 1970), *B. mori* and *Antheraea mylitta* (Hurkadli *et al* 1988c). In the present investigation, a moderate Δ^5-3β-HSDH activity obtained in the lamellar epithelial cells of ovarian cap of larval and pupal stages and a weak activity obtained in the follicle epithelial cells of third stage follicle and a moderate activity in the follicle epithelial cells, nurse cells and intermediate cells during 4th, 5th and 6th follicle growing stages may indicate the ability of these tissues to metabolize DHA to androstenedione and pregnenolone to progesterone. The absence of Δ^5-3β-HSDH activity in the oocyte-nurse cells complex and ovariolar sheath in stage 3 follicles may indicate that they are not able to metabolize DHA and pregnenolone during this stage. The activity in traces obtained in the matrix cells during larval and pupal period may indicate its ability to metabolize these steroids to some extent.

After the initiation of vitellogenesis oocyte showed Δ^5-3β-HSDH activity in the peripheral region of the ooplasm. This observation indicates that oocyte attains ability to metabolize DHA and pregnenolone only after the commencement of vitellogenesis but not prior to it. The absence of Δ^5-3β-HSDH activity in the interfollicle stalk cells suggest that they do not have the capacity to metabolize these steroids. The presence of Δ^5-3β-HSDH activity in the precursors cells in the germarium, the immature and mature oocytes in the ovarioles of adult and in the atretic follicles of *P. ricini* suggest their ability to metabolize DHA and pregnenolone.

The gonadal and non-gonadal tissues of the cricket, *G. domesticus* are known to transform testosterone to androstenedione (Lehoux and Sandor 1969). In the ovary of *B. mori* estradiol was extracted, identified and estimated by radioimmunoassay and chromatography-mass spectrometry (Ohnishi *et al* 1985) and its role in the ovarian maturation has also been studied (Ogiso and Ohnishi 1986). 17β-HSDH has been histochemically demonstrated in the ovary of *B. mori* and *A. mylitta* (Hurkadli *et al* 1988c).

The presence of 17β-HSDH activity in the various developing and adult ovarian components of *P. ricini* indicate their ability to bring about the interconversions of testosterone to androstenediol and 17β-estradiol to estrone.

11β-HSDH is known to transform 11β-hydroxysteroids to 11-ketosteroids. Amongst insects, prothoracic glands of water beetle, *D. marginalis* is known to produce 11-deoxycorticosterone (Schildknecht *et al* 1966). The presence of 11β-HSDH has been shown in the ovary of *B. mori* and *A. mylitta* (Hurkadli *et al* 1988c). The presence of 11β-HSDH in the ovaries of larva, pupa and adult *P. ricini* and atretic follicles indicate their ability to metabolize 11β-hydroxysteroids.

G-6-PDH is involved in generating reduced nicotinamide adenine dinucleotide phosphate (NADPH) in steroid synthesizing or metabolizing tissues of vertebrates (Mc Kerns 1968). The intensity of G-6-PDH reaction was more than that of Δ^5-3β-HSDH, 17β-HSDH and 11β-HSDH activity. G-6-PDH was also noticed in the interfollicle stalk cells, where the activity of HSDH was not present. From the present investigation it may be inferred that G-6-PDH has wider distribution

compared to HSDH. Further its presence in the ovarian cells that also possess steroid converting enzymes provides an additional evidence that the ovary of *P. ricini* has the potential to convert hydroxysteroids to ketosteroids. The study also confirms the findings of earlier workers (Lehoux and Sandor 1970; Schoenmakers and Voogt 1980; Ohnishi *et al* 1985; Ogiso and Ohnishi 1986; Hurkadli *et al* 1988c) that the so called vertebrate steroids are not the exclusive features of vertebrate gonads but also found in many invertebrates.

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Sodium coupled nutrient transport in the midgut tissue of the silkworm, *Bombyx mori* L.

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Abstract. Fifth instar larvae of *Bombyx mori* were administered with additional Na^+ ion orally. The concentration of Na^+ increased and that of K^+ decreased significantly in the haemolymph within 24 h. There was no marked difference in the ionic concentration of the midgut cell while K^+ increased in the gut content. The protease activity increased significantly in gut lumen with a resultant increase in amino acid concentration, which showed a decrease in gut tissue and haemolymph. The protein content did not show significant change in gut lumen, while in gut tissue and haemolymph it decreased markedly. The results are discussed in relation to the Na^+-linked nutrient transport mechanism in the gut epithelium of *Bombyx mori*.

Keywords. *Bombyx mori*; midgut; nutrient transport; proteases.

1. Introduction

In insects, absorption of nutrients depends on 3 important factors, namely composition of the diet, functioning of nutritive enzymes and membrane permeability of intestinal epithelium (Treherne 1958; Primor and Zlotkin 1980; Harvey 1982). Composition of mulberry leaf (Ito and Kobayashi 1978) and the activity of digestive enzymes in *Bombyx mori*, have been studied in detail (Ito 1978; Sarangi 1986). Further, the food of phytophagous insects is rich in K^+ which moves passively down chemical gradient into haemolymph (Harvey *et al* 1975) and often the nutrients are absorbed from the intestinal lumen, much faster than can be explained. However, very little information is available regarding the effect of Na^+ on the activity of digestive enzymes and the absorption of nutrients by the gut of silkworms. Earlier studies on nutrient absorption in silkworm *B. mori* have confirmed the presence of carrier mediated transport (Sacchi and Giordana 1980) and also active transport (Sacchi *et al* 1981) mechanisms. In the present study, an attempt has been made to observe the influence of Na^+ on the activity of midgut protease and the absorption and transport of proteins, amino acids and ions by the gut of the silkworm *B. mori*.

2. Materials and methods

Bivoltine (NB_{18}) silkworm race was maintained under standard laboratory conditions at 25–28°C and a relative humidity of 70–90% on mulberry leaves (M5 variety). The fifth instar larvae were divided into two batches. Batch I served as control and was fed with the normal leaves. Pilot experiments showed that higher concentrations of NaCl was phagorepellent. Therefore 2% NaCl was sprayed on mulberry leaves and air dried before feeding to the experimental larvae (Batch II) on the 2nd day and all the observations were made after 24 h of feeding.

For ionic determination, the haemolymph was collected in a test tube by cutting the caudal horn of the silkworm larva and was diluted with distilled water (1:19 times). Then the midgut was excised and the gut tissue and gut content were separated. The gut content was centrifuged at 1000 *g* for 5 min and the supernatant was diluted with distilled water (1:10 times). The gut tissue was made to a 10% (w/v) homogenate in distilled water, centrifuged at 1000 *g* for 5 min and the supernatant was diluted with 0·6 N $HClO_4$ (1:1 times). Na^+ and K^+ were assayed by using systronics digital flame photometer.

For the enzyme studies, the midgut was excised along with the contents after freezing the animals for about 12 h at −20°C. The midgut tissue and the gut contents were separately taken for the measurements of protease activity. A 10% (w/v) homogenate of both the materials was prepared in ice-cold borate buffer (pH 11), centrifuged in an IEC refrigerated centrifuge at 1000 *g* for 15 min and the supernatant was used as the enzyme source. Protease activity was measured according to Eguchi and Iwamoto (1982) with slight modification that the pH was 11 instead of 11·2. Protein concentration was determined according to the method of Lowry *et al* (1951). Three ml of tissue homogenate or haemolymph were deproteinised by addition of 80% methanol, the supernatant was collected after centrifugation at 1000 *g* for 10 min and used for the estimation of free amino acids (Moore and Stein 1954). Four to five larvae were taken for each determination during each spraying and the average value of 4 determinations along with standard deviation is presented (figures 1–3). Student's '*t*' test was conducted to assess the level of significance.

3. Results

The larvae accepted the mulberry leaves sprayed with 2% NaCl. Extra sodium in the experimental larval diet resulted in the increase in Na^+ and decrease in K^+ concentrations in the haemolymph significantly ($P = < 0·001$). Although the gut content and gut tissue in experimental larvae showed a change in ionic composition, it was not significant except for a notable increase ($P = < 0·005$) in K^+ concentration in the gut content (figure 1).

Figure 2 shows the effect of Na^+ on total free amino acids and total proteins of the gut content, gut tissue and haemolymph of the fifth instar larva of *B. mori*. The

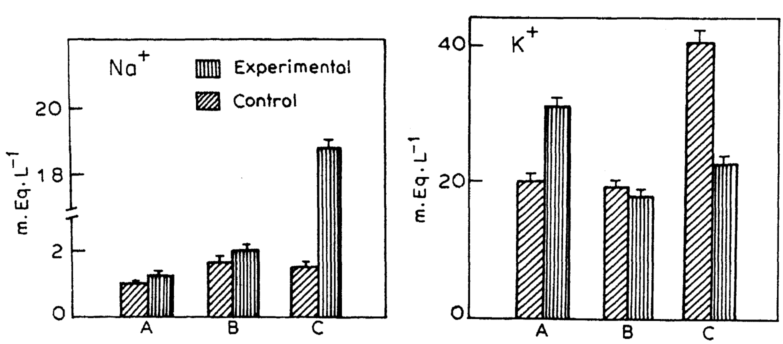

Figure 1. Effect of oral ingestion of Na^+ on the ionic composition of control and experimental larvae of *B. mori*. (A), Gut content; (B), gut tissue; (C), haemolymph.

concentration of total amino acids in the gut content increased 2·7-fold, while in both gut tissue and haemolymph it decreased following the administration of Na^+. The concentration of protein almost showed a similar trend as that of the amino acid content. However, the increase in the concentration of protein in gut content was not significant.

Figure 3 shows the activity of protease in the gut content and gut tissue of the fifth instar silkworm larva. In general, the protease activity was found to be 8–9 times higher in gut content compared to gut tissue. Oral administration of Na^+, though slightly decreased the protease activity in gut tissue, had a significant ($P = < 0{\cdot}05$) influence on the enzyme activity by increasing its level in the gut content.

4. Discussion

Active transport mechanism has been postulated to account for Na^+ absorption and its concentration in the haemolymph of the silkworm *B. mori* (Sacchi *et al* 1981) and in other insects as well (Shaw 1955; Farmer *et al* 1981). Barret (1982) has shown that Na^+ uptake could be mediated by a linked Na^+/K^+ pump studied at basal or

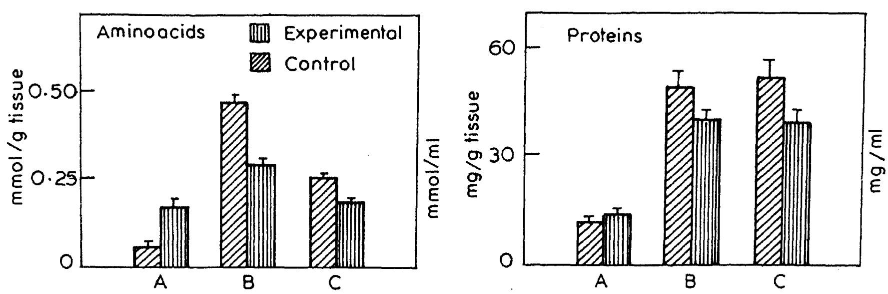

Figure 2. Effect of oral ingestion of Na^+ on protein and amino acid concentration of control and experimental larvae of *B. mori.* (A), Gut content; (B), gut tissue; (C), haemolymph.

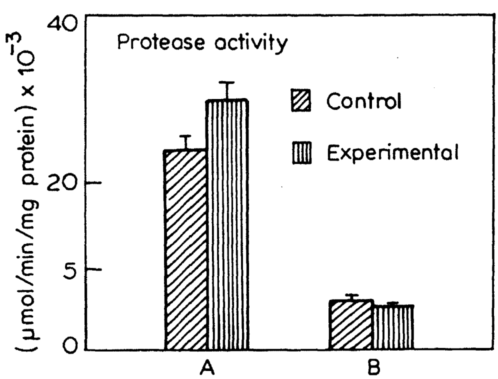

Figure 3. Effect of oral ingestion of Na^+ on midgut protease activity of control and experimental larvae of *B. mori.* (A), Gut content; (B), gut tissue.

lateral surface of the midgut cell. The present results show an increased Na^+ concentration in the haemolymph which might be due to its transport across the epithelial membrane into haemolymph. Further, to maintain the neutrality of haemolymph the K^+ concentration is brought down significantly. Relative lack of change in the ionic concentration of epithelial cells suggests that many substances cross the intestine without being accumulated in the cellular epithelium. The difference in the ionic composition in two compartments separated by midgut follows the same trend as reported earlier in *B. mori* (Giordana and Sacchi 1978). The increased K^+ concentration in gut content can be related to an active extrusion of K^+ across the goblet cells of epithelial membrane into the intestinal lumen in lepidopteran larvae as suggested by Harvey (1982). It is clear from the results that oral administration of Na^+ stimulates the release of midgut protease into the gut lumen. As a result, active hydrolysis of dietary proteins occurs which might partly contribute to the increase in the amino acid concentration in the gut content. But interestingly the concentration of proteins in the gut content does not show a significant change. As, the general food consumption did not show any apparent change in the experimental set, this requires further investigation to draw a conclusion that the total nitrogen uptake (proteins and free amino acids) by the larva increases under the influence of Na^+. It has been reported that addition of amino acids into the lumen increased the trans-epithelial potential difference in silkworms (Giordana and Sacchi 1978) and co-transport of some of the amino acids in the presence of K^+ or Na^+ is observed from lumen to the gut tissue (Giordana *et al* 1982). Thus an increased accumulation of amino acid in gut lumen as a result of Na^+ administration, might influence the transport of additional amount of amino acids across the epithelial membrane of the gut. Further, the decrease in the concentration of proteins and amino acids in the gut tissue and the haemolymph of the experimental larvae indicates that there exist a possible Na^+ mediated transport mechanism that facilitates the transport of nutrients from the gut tissue possibly to other tissues like silk gland and fat body during the fifth instar larval development of the silkworm, *B. mori*.

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Caryophylliasis in the catfish, *Clarias batrachus* L.: some histopathological observations

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Abstract. The extent of damage to the intestine caused by the caryophyllid cestodes, *Lytocestus indicus, Djombangia penetrans*, and by multiple infections comprising several species of caryophyllids in the cat fish, *Clarias batrachus* L. is studied. *Djombangia penetrans* produced large nodules in the intestinal wall. *Lytocestus indicus*, being less deeply penetrative, caused ulceration of the affected tissue, with hyperplasia of the muscularis in severe cases of infection. Pathogenicity due to multiple infections was minimum, apparently limited to denudation of the mucosal folds.

Keywords. *Caryophylliasis*; *Clarias batrachus*; *Lytocestus indicus*; *Djombangia penetrans*; histopathology.

1. Introduction

Cestodes, caryophyllids in particular, are well known to produce certain adverse effects on their piscine hosts (Amlacher 1961; Musselius *et al* 1963; Akhmetova 1966; Mackiewicz *et al* 1972; Bauer *et al* 1973). These include mechanical blockage of the gut lumen (Scherban 1965), production of lesions (Mackiewicz *et al* 1972; Hayunga 1979a, b) and in the physiological state of the host thereby predisposing it to other infections (Jara and Szerow 1981a, b; Kadav and Agarwal 1982, 1983a, b).

In the Indian subcontinent though the dominant hosts for caryophyllids are the siluriform and cypriniform fishes, *Clarias batrachus* (L.) and *Heteropneustes fossilis* (Bloch) are the chief hosts (Mackiewicz 1981). During our studies on the helminth parasites of fishes in north-east India, *C. batrachus* was found to be frequently and mainly parasitized by the caryophyllids, *Djombangia penetrans* Bovien 1926; *Lytocestus indicus* (Moghe 1925) Woodland 1926 and to a lesser extent by other species of *Lytocestus* such as *L. filiformis* Fuhrmann and Baer 1925, *L. birmanicus* Lynsdale 1956 and *L. longicollis* Rama Devi 1973 which occurred concurrently with the dominant species, and formed multiple infection.

In the present study, histopathological changes in the intestinal wall of the host subsequent to infections by these parasites in single or multiple infection are reported.

2. Materials and methods

The intestinal tissue, with parasite *in situ*, was fixed in Bouin's fixative, and processed for routine histological investigations. Paraffin sections of 6–7 μm thickness were stained following Mallory-Heidenhein's triple staining technique. Sections of intestinal tissue from uninfected host fish served as controls.

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3. Results

The normal intestinal wall (figure 1) of *C. batrachus* is composed of mucosa (columber epithelium consisting of absorptive and mucus secreting cells), submucosa (highly vascularized and extending into the villi as lamina propria), muscularis (comprising inner circular and outer longitudinal muscle fibres) and the outermost coat, serosa, with a rich vascular supply (Khanna 1980).

With *D. penetrans*, *L. indicus* and caryophyllidean-mix infections the intestinal wall revealed different pathological changes.

3.1 *L. indicus*

This species penetrates deep into the muscularis layer (figure 2). It was observed that despite this there was no nodule formation. At the site of scolex attachment to the intestinal wall mechanical displacement and compression of tissue layers such as mucosa, submucosa and muscularis were noticed. Due to excess pressure exerted by the scolex, in severe cases the submucosa became hyperplastic (figure 3). No leukocytic infiltration was observed; however, in some cases a thin mucoid interface between the host-tissue and the scolex was observed. Loosening of muscle fibres was also evident. Apart from these changes, infection with 8–10 worms per sq. cm. area seemed to cause some overcrowding effect, resulting in the blockage of the intestinal lumen.

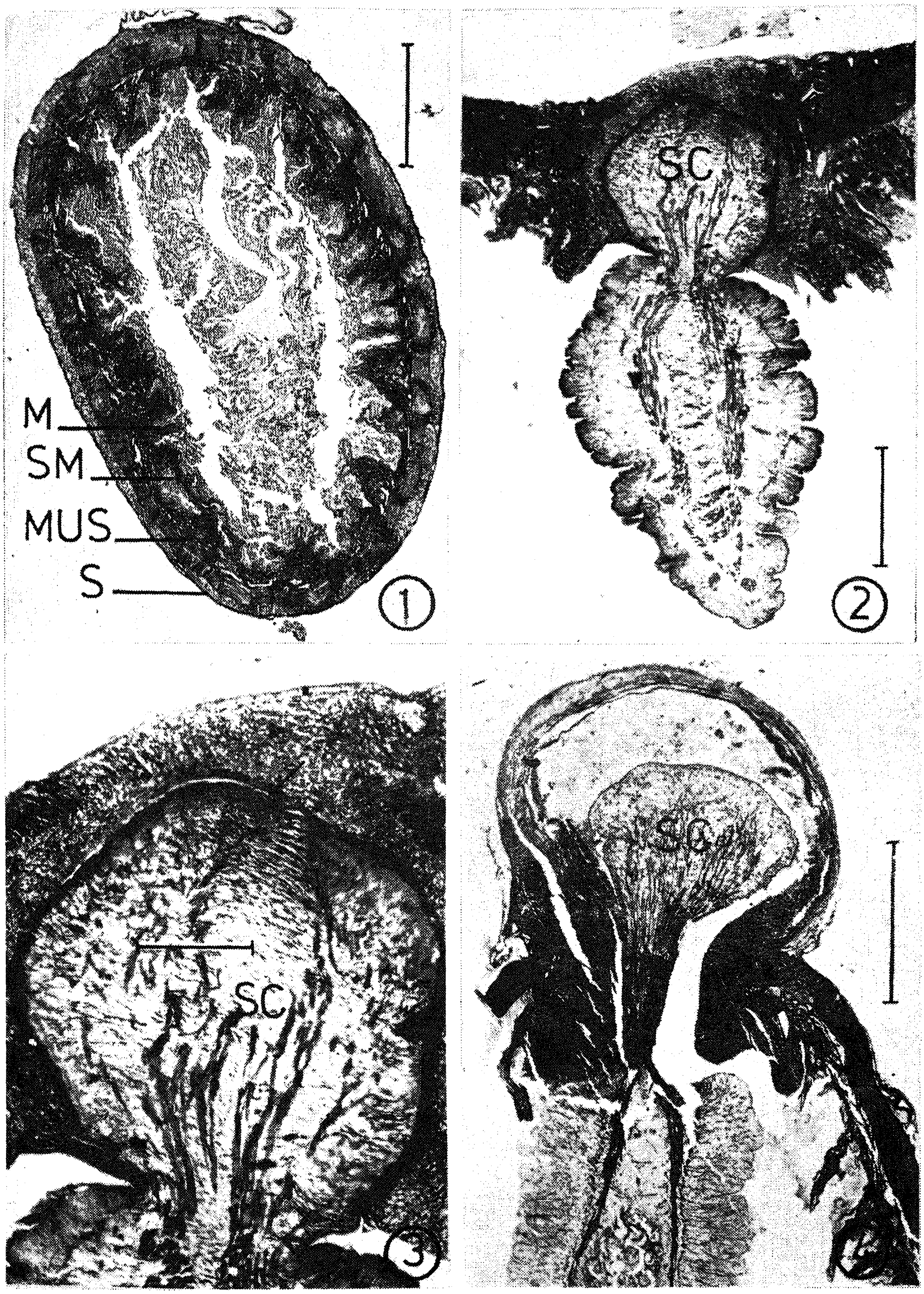
3.2 *D. penetrans*

A sucker at the tip of the worm aids in its firm attachment to the host's tissue. Frequently nodular swellings were observed on the outer wall of the intestine, caused by the penetration of the parasite into the serosa. Formation of nodules led to the production of tunnels through the intestinal wall in which the neck portion of the worm remained embedded. While the scolex was encapsuled in the lumen of the nodule, the body remained free in the lumen of the intestine. As a result of compression of the mucosal folds, the submucosa and the muscularis formed plaques at the site of attachment. The compressed tissue layers appeared thickened on the two sides of the tunnel. However, the nodular lining did not show such thickening. Each nodule contained only one worm, but debris and necrotic tissue were seen near the scolex. Besides, an interface layer separated the host's tissue from the holdfast of the worm (figure 4).

Like *L. indicus*, *D. penetrans* also caused mechanical blockage of the intestinal lumen.

3.3 *Multiple infections of caryophyllids*

Generally, multiple infection comprised as many as 3 to 4 different species and a total of 10–12 worms concurrently parasitised a single host. Most of these worms had elongated bodies and hence occupied a considerable niche length resulting in mechanical obstruction of the gut. The pathogenicity was not severe because no ulceration or nodular formation took place in the host's intestinal wall, but the intestinal villi or the mucosal folds got compressed.



Figures 1–4. Histopathology of the host tissue. **1.** Normal intestinal tissue of *C. batrachus* showing the different layers. **2.** *L. indicus* anchored to the intestine, illustrating penetration up to the muscularis layer (scale bar = 0·5 mm). **3.** The same at higher resolution showing hyperplasia of the muscularis and a thin interface layer (arrow) (scale bar = 0·15 mm). **4.** Section through a nodule harbouring the scolex of *D. penetrans*. Note the muscularis thickening adjacent to the tunnel (arrow) (scale bar = 0·5 mm).
(M, Mucosa; SM, submucosa; MUS, muscularis; S, serosa; Sc, scolex).

4. Discussion

The distribution of *L. indicus*, *D. penetrans* and species constituting the multiple infection within the host's intestine shows a definite habitat preference depending upon the type of scolex. While *L. indicus* and *D. penetrans* inhabited the duodenum, species such as *L. birmanicus*, *L. longicollis* and *L. filiformis* were always found in the anterior and posterior loops of the intestine.

The effects on the host seem to be related to the mode of attachment of the scolex (Mackiewicz *et al* 1972; Bauer *et al* 1973). Thus, deep penetrating scoleces of *L. indicus* and *D. penetrans* caused serious damage to the host's intestinal wall.

L. indicus produced shallow ulcers within the host's tissue causing destruction of villi, mucosa and submucosa. These observations are in conformity with those of Satpute and Agarwal (1974), Ahmed and Sanaullah (1979), Bose and Sinha (1981) and Niyogi and Agarwal (cited from Agarwal 1985). However, *L. indicus* was never found to reach the serosa and no leukocytic infiltration was observed. Further, the pinocytotic vesicles in the holdfast end of the worm as observed by Niyogi and Agarwal (cited from Agarwal 1985) were also found lacking. Since no degenerated tissues were seen within the folds of the scolex end, these (folds) could not be considered pinocytotic vesicles. The pronounced tissue reaction expressed as hyperplasticity of the submucosal layer may probably be attributed to the secretion of the gland cells that are present in the holdfast end (unpublished results). Such gland cells (='Frontaldrüsen' and 'Faserzellenstränge') are reported to be present in the scolex and neck region of many caryophyllids (Janizewska 1954; Richards and Arme 1981) and assist in attachment (Hayunga 1979a) or penetration of the host's tissue by secreting lytic enzymes (Slais 1961). The highly developed musculature in the holdfast end may also be responsible for exertion of pressure in the tissue layers leading to their compression. However, the mechanical obstruction is caused due to the occurrence of parasites in clusters.

The pathogenicity caused by *D. penetrans* can be compared to that by other caryophyllids viz., *Hunterella nodulosa*, *Biacetabulum biloculoides*, *Monobothrium ulmeri* and *M. ingens* which also produce pronounced host reaction in the form of nodules (Mackiewicz *et al* 1972). Lacking a specialized holdfast, *H. nodulosa*, like *D. penetrans*, produces a large nodular thickening with a pronounced active chronic inflamatory reaction. However, the nodular lining of *D. penetrans* appears to be necrotic with debris in the pit and the nodule is single chambered, harbouring only one worm unlike the nodules of *H. nodulosa* which some times have more than one chamber containing as many as 127 worms up to various stages of development (Mackiewicz and McCrae 1962). Debris and necrotic tissue surrounding the scolex are also found in the nodules formed by *B. biloculoides*, *M. ingens* and *M. ulmeri*. However, the size of the nodule produced by *D. penetrans* appears to be the smallest. Histologically, the nodules of *D. penetrans* appear similar to those produced by *Polyonchobothrium clarias*, a pseudophyllidean in the gall bladder of *Clarias mossambicus* (Wabuke-Bunoti 1980), in having 3 layers namely, the continuous outer epithelial layer, a granulomatous sub-epithelial layer and the innermost fibrous connective tissue layer; however the last-mentioned two layers are not highly vascularized.

While Mackiewicz *et al* (1972) associated the changes with proteolytic secretions of the frontal glands, Hayunga's (1979b) observations on *H. nodulosa* revealed no evidence of proteolysis or necrosis of host tissue. In his opinion, probably the eosinophilic matrix was primarily an adhesive layer which acted as a strong irritant, and the latter combined with a strong contraction of the worm caused ulceration and loss of epithelium. However, the occurrence of necrotic debris in the present study is suggestive of the presence of proteolytic enzymes in the holdfast end. This needs further investigation.

Another remarkable difference between the host reaction produced by *D. penetrans* and that by other nodule-forming caryophyllids is that in none of the latter does there occur a muscular thickening on the sides of the penetration site. As a result of penetration or burrowing deep up to the serosa by *D. penetrans* the tissue layers, across which it penetrates, get thickened. Similar observations were made by Satpute and Agarwal (1974) and Ahmed and Sanaullah (1979) on *Djombangia* spp. infections in *C. batrachus.* The nodule formation which seems to be an inflamatory response of the host provides a sheltered habitat and firm anchorage to the worm. This host response is an example of exploitation by the parasites of its host defence mechanisms to its own advantage (Hayunga 1979a).

The pathological changes manifested in the form of shortening of intestinal villi or compression of the mucosal folds due to multiple infection are probably a consequence of pressure necrosis. Except the alterations in the absorptional area of the intestine, and mechanical blockage of its luminal space, no serious host reaction is produced due to multiple infection. Multiple infections of caryophyllids are common (Mackiewicz *et al* 1972) and seemingly have no detrimental effect on the host (Agarwal 1985).

The present study also suggests that the mode of attachment is primarily responsible for triggering significant host reaction. While single worm of *L. indicus* or *D. penetrans* results in shallow ulcers or nodule formation, respectively, multiple infection results only in the atrophy of the intestinal villi, and the host reaction elicited is minimum, probably because the mode of attachment of the worms is very weak.

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Insecticide induced hematological changes in pigeons

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Abstract. Hematological responses of bluerock pigeon (*Columba livia* Gmelin) were studied after oral administration of chlordane (a cyclodiene), fenitrothion (a phosphothioate) and carbaryl (a carbamate) for one week. Comparable hematological disorders were induced by these insecticides which include reduction in total count of peripheral erythrocytes, hemoglobin content, hematocrit and total cellularity of spleen. Total count of peripheral leucocytes, on the otherhand, increased with marked heterophilia together with lymphopenia and monocytopenia. Both bleeding and clotting time became conspicuously prolonged in the experimental birds. The results indicate potential to use hematological responses for rapid on the spot assay of insecticide toxicity in non-target animals.

Keywords. Hematology; anemia; insecticide; spot-assay; pesticide monitoring.

1. Introduction

Abundant use of insecticides in the field has indeed posed serious risks to wild life, particularly for the more sensitive animals like birds (Stickel 1973). India is one of the largest users of agricultural insecticides in recent times (Allen *et al* 1984). Of the organic insecticides used in India, organochlorines constitute the largest group, followed by phosphatic and carbamate compounds (Anon 1984).

We have been studying the feasibility of using hematological responses to predict early warning of insecticide-toxicity in non-target animals. Pigeon is found to be quite sensitive to insecticides (Mandal 1986; Mandal and Lahiri 1985). The present paper reports hematological responses of pigeons following short duration exposure to selected insecticides representing 3 principal classes.

2. Materials and methods

Sexually mature bluerock pigeon (*Columba livia* Gmelin) of either sex trapped from the same geographical areas were procured locally during the months of March and April. They were acclimatized to laboratory conditions atleast for 7 days and were maintained on adequate balanced diet (Chakrabarti 1986). In pilot experiments dietary influence was investigated and no variation in hematological parameters was noticed with the balanced diet supplied to the birds. Five birds were kept in each cage. The cages were sufficiently large to allow the pigeons a reasonable freedom of movement. Ten birds were evenly divided into control and experimental groups for each set of experiment. Olive oil suspension of fenitrothion [0 0-dimethyl-0(3-methyl-4-nitrophenyl) phosphothioate], chlordane (β-cis-octa chlorometheno-tetrahydroindane) and carbaryl (1-naphthyl-N-methyl-carbamate) were administered intragastrically twice per week for one week only, as per the following schedule (Mandal 1986):

Control — Vehicle only
Group I — Chlordane 5 mg/kg body wt
Group II — Fenitrothion 0·1 mg/kg body wt
Group III — Carbaryl 0·1 mg/kg body wt

Following termination of experiment after one week, blood samples were collected from the pectoral vein in tubes previously rinsed in heparin for analyses of hematological indices. The birds were then killed by decapitation and the spleens were quickly dissected out for total count of cellularity. Peripheral blood counts of red blood cells (RBC) and white blood cells (WBC) were done with a hemocytometer by the method of Natt and Herrick (1952). Bleeding time (BT) was recorded according to Duke's method (Kolmer *et al* 1969). Clotting time (CT) was measured by the capillary glass tube method (Kolmer *et al* 1969) and haemoglobin (Hb) was determined by the Hellige method (Halaz 1967). Avian blood cells settle very slowly and therefore to determine erythrocyte sedimentation rate (ESR), sedimentation tubes (Westergren) were positioned at 45° (Washburn and Meyers 1957), thereby substantially increasing the sedimentation rate. Hematocrit (hct) was determined by microhematocrit method (Wintrobe *et al* 1976). Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated from the RBC count, hemoglobin content and hematocrit (Dacie and Lewis 1975). WBC differential counts were made from Wright stained blood smears (Lucas and Jamroz 1974). The number of nucleated cells per spleen was determined with a hemocytometer after mincing the organ in cold phosphate-buffer saline and sequential passage through 20–, 22– and 23 gauge needle. Since avian spleen produces only lymphocytes (Payne 1971; Hodges 1974), differential counts of spleen were not done. Statistical analyses were done by Student's '*t*' test.

3. Results

The birds remained active and healthy throughout the experiment. Apetite and food intake was normal and body weight remained unaltered in the experimental birds. There was no pathological symptoms to suggest any overt toxicity due to insecticide administration.

Results of hematological studies have been presented in tables 1 and 2. Mild to moderate changes of hematological indices were observed in the pigeons following intake of all the 3 insecticides. It is evident from table 1 that in all the groups of birds significant reduction of total peripheral RBC, Hb content and hct was noticed. Also, ESR and MCV remained unchanged in these birds. MCH and MCHC were reduced in chlordane and carbaryl-fed pigeons. However, there was significant increase in the total counts of peripheral WBC in the insecticide fed pigeons. Simultaneously, prolongation of both bleeding time and clotting time was observed in such birds. Differential counts of formed elements in the experimental pigeons is characterised by both marked heterophilia and significant lymphopenia and monocytopenia (table 2). Eosinophilia was observed only in chlordane and carbaryl-fed pigeons. Total splenic cellularity was reduced significantly in all the insecticide fed birds, though there was no significant change of weight of this organ (table 3).

Table 1. Hematological indices of the whole blood of control and insecticide-fed pigeons.

	Control	Chlordane	Control	Fenitrothion	Control	Carbaryl
Total count of RBC ($10^6/\mu l$)	2·60 ± 0·11	2·20 ± 0·10^{b}	2·66 ± 0·05	2·25 ± 0·16^{b}	2·60 ± 0·11	2·20 ± 0·10^{b}
Hemoglobin (g/dl)	12·80 ± 0·50	9·02 ± 0·88^{a}	14·13 ± 0·34	12·01 ± 0·76^{b}	12·80 ± 0·50	9·02 ± 0·88^{a}
Hematocrit (%)	38·33 ± 1·20	32·25 ± 1·85^{b}	35·16 ± 0·75	30·00 ± 1·40^{b}	38·33 ± 1·20	32·25 ± 1·85^{b}
ESR (mm/h)	40·50 ± 1·85	37·16 ± 1·50^{NS}	42·10 ± 1·30	39·00 ± 1·03^{NS}	40·50 ± 1·85	37·16 ± 1·50^{NS}
MCV (fl)	147·42 ± 1·11	146·60 ± 0·98^{NS}	132·18 ± 4·10	133·33 ± 2·20^{NS}	147·42 ± 1·11	146·60 ± 0·98^{NS}
MCH (pg)	49·23 ± 2·21	41·00 ± 2·32^{b}	53·12 ± 1·40	53·37 ± 1·22^{NS}	49·23 ± 2·21	41·00 ± 2·32^{b}
MCHC (g/dl)	33·39 ± 1·35	27·96 ± 1·40^{b}	40·19 ± 1·60	40·03 ± 0·87^{NS}	33·39 ± 1·35	27·96 ± 1·40^{b}
BT (s)	50·25 ± 4·28	70·83 ± 3·53^{a}	48·83 ± 2·53	58·00 ± 2·65^{b}	50·25 ± 4·28	70·83 ± 3·53^{a}
CT (s)	26·75 ± 1·18	31·00 ± 1·24^{b}	30·66 ± 1·86	36·00 ± 1·26^{b}	26·75 ± 1·18	31·00 ± 1·24^{b}

Values are mean ± SE. *P* values: $^{a}<0·01$, $^{b}<0·05$. NS, Not significant.

Table 2. Total and differential count of WBC in the whole blood of control and insecticide-fed pigeons.

	Control	Chlordane	Control	Fenitrothion	Control	Carbaryl
Total count of WBC ($10^3/\mu l$)	15·75 ± 0·58	18·30 ± 0·88^{b}	15·90 ± 0·73	17·68 ± 0·24^{b}	15·75 ± 0·58	18·30 ± 0·88^{b}
Differential count (%)						
Lymphocyte	63·60 ± 2·44	54·55 ± 2·10^{b}	62·20 ± 1·62	52·90 ± 2·50^{b}	63·60 ± 2·44	54·55 ± 2·10^{b}
Monocyte	2·53 ± 0·13	2·10 ± 0·10^{b}	3·10 ± 0·20	2·30 ± 0·10^{a}	2·53 ± 0·13	2·10 ± 0·10^{b}
Heterophil	26·89 ± 2·15	35·54 ± 1·40^{a}	27·52 ± 2·10	37·24 ± 1·60^{a}	26·89 ± 2·15	35·54 ± 1·40^{a}
Eosinophil	5·80 ± 0·25	6·79 ± 0·32^{b}	6·18 ± 0·35	6·70 ± 0·20^{NS}	5·80 ± 0·25	6·79 ± 0·32^{b}
Basophil	1·18 ± 0·09	1·02 ± 0·01^{NS}	1·00 ± 0·06	0·86 ± 0·05^{NS}	1·18 ± 0·09	1·02 ± 0·01^{NS}

Same notations as in table 1.

Table 3. Total cell count and weight of spleen of control and insecticide-fed pigeons.

	Control	Chlordane	Control	Fenitrothion	Control	Carbaryl
Total count of splenic cells (10^9/spleen)	3·00 ± 0·10	2·05 ± 0·24^a	3·09 ± 0·15	2·59 ± 0·11^b	3·00 ± 0·10	2·05 ± 0·24^a
Weight of spleen (mg)	417·50 ± 7·61	391·20 ± 8·70^{NS}	418·20 ± 20·14	400·33 ± 19·30^{NS}	417·50 ± 7·61	391·20 ± 8·70^{NS}

Same notations as in table 1.

4. Discussion

Organic insecticides belonging to different classes induced more or less similar hematologic disorders in pigeon even when administered for short duration. The most common response being the development of mild to moderate anemia as evidenced by significant reduction of total count of RBC, Hb content and hct in the insecticide-fed birds. Reduction in Hb content can be attributed to the decreased RBC number. While Hb content and hct decreased in the insecticide-fed pigeons, there was no marked change in MCV. MCH and MCHC decreased only in chlordane and carbaryl-fed pigeons (table 1). It thus appears that anemia is secondary to possible accelerated hemolysis, hemorrhage and/or reduced erythropoiesis inflicted by these insecticides in the pigeons. Similar effects have also been found in birds chronically exposed to insecticides (Mandal and Lahiri 1985; Mandal 1986). However, the mechanism of anemia in fenitrothion-fed pigeons might differ from other two groups since MCH and MCHC did not change as in chlordane or carbaryl-fed birds.

Normally in pigeons, lymphocytes constitute approximately 62% of the WBC population. Concomitant with increase in the total number of circulating leucocytes in the insecticide-fed pigeons, differential counts of these formed elements have undergone a shift from lymphocytosis to heterophilia. Such virtual reversal of lymphoid-myeloid ratio in the insecticide-fed pigeons possibly indicates systemic reaction to these chemicals. In birds both lymphocytes and monocytes are formed in the spleen (Lucas and Jamroz 1974); thus observed lymphopenia and monocytopenia in the experimental birds possibly result from decreased cellularity of the spleen (table 3).

There was consistent prolongation of both bleeding and clotting time in such birds. Increase in clotting time was reported in rats (Srinivasan and Radhakrishnamurthy 1983), humans (Barsel'yants 1969), mice (Gupta *et al* 1983), pigeons and other birds (Mandal and Lahiri 1985; Mandal 1986), particularly after long exposure to various insecticides. It is evident from the present study that all the 3 classes of insecticides are quite hematotoxic and evoke common disorders in pigeon even when exposed for relatively short duration. Comparable hematologic response was reported for rather longer exposure to such chemicals (Mandal and Lahiri 1985; Mandal 1986). Hematological responses of pigeons to insecticides thus seem to be quite effective for rapid detection of prepoisoning cases and may be used for on the spot-assay of toxicity of insecticides particularly in the field situation.

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Ontogeny of aggressive and submissive behaviour in free living rhesus monkeys (*Macaca mulatta*)

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Abstract. Twenty rhesus monkeys of various age-sex classes were observed by focal animal and one-zero sampling technique for 12 months at Galta hillocks near Jaipur (Rajasthan). Behavioural elements sampled were screech, scream, bark, threat, chase, clasp-pull, bite, fear grimace, lip-smack and submit. These elements were categorized into aggressive and submissive behaviours by pooling the frequencies of elements. Mean per cent frequencies were derived and analysed by ANOVA, trend analysis, Kruskal Wallis one way ANOVA and Mann-Whitney 'U' test. It was found that submissive behaviour among females and aggressive behaviour among males changed with age. The findings have been interpreted on the basis of social conditions and the typical spatial position of the age-sex class within the group space/structure.

Keywords. Free ranging rhesus monkey; ontogeny; aggression; submission; trend analysis.

1. Introduction

Agonistic behaviour of primates in general and rhesus monkeys in particular is considered to be an important determinant of the organization of inter- and intra-group interactions. Although aggressive encounters occur with comparatively low frequencies (Bernstein and Mason 1963; Hall 1964; Post and Baulu 1978), the ability to handle attacks, displaying threatening and submissive gestures, agonistic aiding and fight interference are common situations in the social behaviour of rhesus monkey groups (Kaplan 1977; Bernstein and Carolyn 1985a). Failure in emitting such responses may lead to very high cost e.g. injury, physical disability, expulsion from the group, losing the rank and even death. Like play, affiliation and dominance, aggressive and submissive behaviours are shaped through socialization (Symons 1978).

Environment has been found to influence general aggressiveness of rhesus monkeys, e.g. urban monkeys are more aggressive than forest monkeys (Singh 1969; Lindburg 1971; Southwick *et al* 1976; Roonwal 1977). Age, sex, rank and kinship may also explain variability of agonistic behaviour in rhesus monkeys (Altmann 1968; Moller *et al* 1968; Hamburg 1971; Bernstein and Carolyn 1985b). Expression of aggressive behaviour is also influenced by internal physiological conditions such as reproductive cycle in females and seasonal changes in male hormones (Wilson and Boelkins 1970; Rowell 1972; Mallow 1981). The present study deals with ontogenetic changes in aggressive behaviours.

2. Methods

2.1 *Study area and group*

One group from a free ranging population of rhesus monkeys in Galta hillocks near

Jaipur (Rajasthan) was observed. Details of the characteristics of habitat, population and the study group have been reported earlier (Singh 1986).

2.2 *Focal animals*

Table 1 describes the number, transitional stage, approximate age, sex and duration of the 20 animals that served as subjects. Two animals of each sex in each of 6 developmental stages (except among young adults) were studied. Two old females were studied but old males could not be identified as age estimations within adult class is not possible for males as they continue to take part in breeding activities. In the sample, 12 animals were so selected that at the beginning they fell in one age class and after 12 months they entered the next age class, thus transition from one age class to the next could be studied. The month of May, the peak birth season, was considered as cut off point to decide age classes, i.e. progeny of the same year was considered 6 months old in November when the observations were started and similarly the progeny of the previous year was labelled as 1·5 years old and so on. Age status among adults was based on reproductive status or parity, dentition and colour of the skin of hindquarters or around callosities as size variation is obscured in the adult stage which is 4/5th of the life span of rhesus monkeys. The age of transition differed between the sexes.

2.3 *Description of recorded behavioural elements*

Seven behaviours and 3 vocalizations belonging to the agonistic category were defined as under:

(i) Screech—A marked rise and fall in pitch in single or extended serial vocalization. Occurs in submissive context.
(ii) Scream—A high pitched lacking rise and fall of screech. Occurs under severe attack.
(iii) Bark—A short, low and harsh vocalization, resembling a cough or louder sound which accompanies the threat.

Table 1. Characteristics of focal animals (n = 20).

Age class	Sex	n	Approximate age during study (yrs)	Animal codes	Duration of observation (h)
Transition-I	Female	2	0·5–1·5	FI_1, FI_2	16 each
Infancy to juvenilehood	Male	2	0·5–1·5	MI_1, MI_2	16 each
Transition-II	Female	2	1·5–2·5	JF_1, JF_2	13·33, 16
Juvenilehood to adolescence	Male	2	3·5–4·5	MJ_1, MJ_2	16 each
Transition-III	Female	2	2·5–3·5	AF_1, AF_2	16 each
Adolescence to adulthood	Male	2	4·5–5·5	SA_1, SA_2	13·33, 16
Within adulthood	Female	1	4·5–5·5	YF (nulliparous)	16
Young adult	Male	1	5·5–6·5	YM (Peripheral)	16
	Female	2	Multiparous (over 8)	MF_1, MF_2	16 each
Mature adult	Male	2	Central, fullsized (over 8)	MM_1, MM_2	16 each
Old	Female	2	Non reproductive (over 20)	OF_1, OF_2	13·33, 16

(iv) Threat—A varied facial communicating pattern, which may include more elements with increasing intensity of expression such as opening the mouth to expose only the lower teeth; thrusting the head forward; flattening the ears against the head; retracting brow; frowning; erection of hair; body is held stiff, upright and barking etc.
(v) Chase—Vigorous following of withdrawee while showing repeated threats and invariable attacks on the opponent upon capture.
(vi) Clasp-pull—Any brief nip, cuff, push, pull with closure of hand.
(vii) Bite—Common usage.
(viii) Fear grimace—A facial expression with lips retracted and teeth tightly clutched, generally accompanied by tenseness of musculature, withdrawal of upper and lower lips to expose teeth.
(ix) Lip smack—Rapid opening and closing of the pursed lips probably accompanied by tongue motions and producing soft clicking sound.
(x) Submit—Assuming motionless rigid posture to reduce the appearance in size in front of another animal.

3.1 *Procedure*

A time ruled check list was used to record codes of the above defined behaviours using focal animal and one zero sampling of behaviour (Altmann 1974). Each subject was observed for two sessions per week of 10 min each for 12 months covering all the social and climatic seasons. Observational sessions were randomly spread over all the diurnal hours from 5 am to 7·40 pm. Aggressive and submissive encounters during artificial feeding, interactions with other species and with animals of other groups were not recorded or scored. The interacting animal's age-sex class was recorded; however, for the infant the younger juvenile sex was not obvious and only age class was recorded. Aggressive encounters involving more than 4 animals were categorized as subgroup encounters and not scored. Due to the death of one juvenile female (JF_1), one old female (OF_1) and the migration of one sub-adult male (SA_1) in transition, 48 observation sessions could not be completed (table 1). The duration of actual recordings for all focal animals was 312 h.

3.2 *Scoring*

Observed frequencies of 10 behavioural elements were grouped into two behavioural categories. Aggressive behaviour included bark, threat, clasp-pull, bite and chase. Submissive behaviour included frequencies of occurrence for screech, scream, fear grimace, lip smack and submit. Since all the behavioural elements (except vocalizations) could be displayed by other animals towards the focal animal, frequencies of 4 behavioural categories were obtained for each animal, viz. submission to, submission by, aggression to and aggression by. To facilitate analysis the mean per cent frequencies for each age sex class were computed in two months age block by using the following formula:

$$\text{Mean per cent frequency} = \frac{\text{Observed frequency}}{\text{Maximum possible frequency}} \times 100.$$

Where, maximum possible frequency = number of animals in age class × number of

observed intervals in 16 sessions (480) × number of interactions in the behaviour element/category.

3.3 *Analysis*

Obtained frequencies were subjected to single factor ANOVA to assess the significance of age changes upon behaviours, separately for each 3 age classes in transition of both sexes. In order to identify the trend or curve function of age and behaviour relationship, the trend analysis for those behavioural categories was attempted where the age had significant bearing on behaviour (Winer 1971). The linear trend hypothesized either an incremental or decremental straight curve function. The quadratic trend predicts the age function in terms of V or inverted V curve, showing incremental trend up to a particular age point followed by decremental trend or vice versa. The cubic trend defines such behavioural patterns which show once incremental then decremental and again taking incremental function or vice versa in a given age period, e.g. 'N' shaped curve. The trend analysis also yields a regression equation of age on behaviour.

Kruskal-Wallis one way ANOVA (Siegel 1956) was used to compare the frequencies among young, mature and old females to test the significance of within class behavioural changes.

Mann-Whitney 'U' test (Siegel 1956), suitable for comparison of two independent samples was used to assess the differences in behaviour between young and mature males.

A descriptive content analysis of the behavioural repertoire was also undertaken to see qualitative changes in terms of first or last occurrence of observed behavioural elements.

4. Results

A general survey of the occurrence of behaviour elements in the observation chart revealed a qualitative pattern in aggressive and submissive behaviours. Table 2 shows the first and last occurrence of the elements with age in both sexes, prepared by putting cross-sections of life span in terms of age classes in continuity. It was

Table 2. Duration of appearance of various behavioural elements during different stages in the life of rhesus monkey.

Behavioural element	Male	Female
Screech	1–3 years	2 years to old age
Scream	Never observed	3 years to old age
Bark	4·5 years to old age	Never observed
Threat	1 year to old age	1 year to old age
Chase	2·5 years to old age	3 years to old age
Clasp pull	1 year to old age	1·5 years to old age
Bite	3 years to old age	4 years to mature adulthood
Fear grimace	1–4 years	1 year to old age
Lip smack	Never observed	1·5 years to old age
Submit	3–4 years	1·5 years to old age

found that some of the behaviours developed during the juvenilehood while others did so during adolescence. At the same time, some of the elements were never observed after a certain age. Sex differences were also evidenced as males assumed an aggressive pattern with age advancement, while females exhibited aggressive as well as submissive behaviours. Adult males were never observed emitting scream and submit elements. Females were never seen barking but displayed all other elements throughout the ontogeny while males continue to display aggressive behaviours.

ANOVA applied to see the significance of age for 4 behavioural categories in both sexes under 3 transitional stages revealed that only 3 F values had associated probability equal to or greater than the confidence level (tables 3–5). It was found

Table 3. Mean per cent frequencies of submission toward others by females in transition stage-I, statistics for ANOVA and trend with regression equation.

Behaviour element	Toward	Mean % frequencies, Age blocks (K = 2 months)						
		1	2	3	4	5	6	Sum
Fear grimace	Female	Nil	Nil	0·05	0·10	0·10	0·05	0·30
	Male	Nil	Nil	Nil	Nil	0·05	Nil	0·05
	Juvenile	Nil	Nil	Nil	0·05	Nil	0·05	0·10
Lip smack	Female	Nil	Nil	Nil	Nil	0·05	Nil	0·05
	Infant	Nil	Nil	Nil	Nil	0·05	Nil	0·05
Submit	Male	Nil	Nil	Nil	0·05	Nil	Nil	0·05
	Female	Nil	Nil	Nil	Nil	Nil	0·05	0·05
Sum		Nil	Nil	0·05	0·20	0·25	0·15	0·65
Average		Nil	Nil	0·007	0·029	0·036	0·021	0·015

Summary of statistics.
(i) Between age blocks $F = 3{\cdot}14$, df $= 5/30$, $P < 0{\cdot}05$.
(ii) Linear trend $F = 16{\cdot}69$, df $= 1/30$, $P < 0{\cdot}01$.
(iii) Regression equation (X = behaviour and Y = age) $X = 0{\cdot}01\ K - 0{\cdot}01$.

Table 4. Mean per cent frequencies of submissive behaviour by females in transition stage-II toward others, statistics for ANOVA and trend with regression equation.

Behaviour element	Toward	Mean % frequencies Age block (K = 2 months)						
		1	2	3	4	5	6	Sum
Fear grimace	Female	0·22	Nil	Nil	0·10	Nil	0·21	0·53
	Juvenile	0·06	Nil	Nil	0·21	0·78	Nil	1·05
	Male	0·06	0·05	Nil	0·10	0·20	0·10	0·51
	Adolescent female	0·06	Nil	0·05	0·05	0·29	0·10	0·55
Lip smack	Female	Nil	0·05	Nil	Nil	Nil	Nil	0·05
	Infant	0·06	Nil	0·05	0·47	0·20	Nil	0·78
Submit	Juvenile	Nil	Nil	0·10	0·05	0·20	Nil	0·35
Sum		0·46	0·10	0·20	0·98	1·67	0·41	3·82
Average		0·066	0·014	0·028	0·14	0·239	0·059	0·091

Summary of statistics.
(i) Between age blocks $F = 2{\cdot}71$, degree of freedom $= 5/30$, $P < 0{\cdot}05$.
(ii) Cubic trend $F = 8{\cdot}87$, df $= 1/30$, $P < 0{\cdot}01$.
(iii) Regression equation (X = behaviour and Y = age) $= X = 0{\cdot}02\ K + 0{\cdot}02$.

Table 5. Mean per cent frequencies of aggression by others toward males in transiti stage-II, statistics for ANOVA and with regression equation.

Behaviour element	By	Mean % frequencies						
		1	2	3	4	5	6	Sum
Threat	Male	Nil	Nil	Nil	Nil	0·10	Nil	0·10
	Juvenile	0·06	0·10	Nil	Nil	Nil	Nil	0·16
	Subadult male	0·11	Nil	0·16	0·05	0·06	0·21	0·59
	Female	0·06	Nil	Nil	Nil	Nil	Nil	0·06
	Infant	0·06	0·10	0·05	0·16	0·23	Nil	0·60
	Juvenile male-1	Nil	0·05	0·05	Nil	Nil	0·10	0·20
	Adolescent female	Nil	0·05	0·05	0·05	0·20	0·11	0·46
Chase	Female	Nil	Nil	0·05	Nil	Nil	Nil	0·05
	Juvenile	Nil	0·05	Nil	Nil	Nil	Nil	0·05
Clasp	Male	Nil	Nil	0·05	Nil	Nil	Nil	0·05
	Juvenile	Nil	0·10	Nil	Nil	Nil	0·42	0·52
	Female	Nil	Nil	0·10	0·21	Nil	0·21	0·52
Bile	Female	0·22	Nil	Nil	0·21	Nil	Nil	0·43
	Juvenile	Nil	Nil	Nil	0·10	Nil	Nil	0·10
Sum		0·51	0·45	0·51	0·78	0·59	1·05	3·89
Average		0·036	0·032	0·036	0·056	0·042	0·075	0·04

Summary of statistics.

(i) Between age block $F=3{\cdot}40$, df$=5/70$, $P<0{\cdot}01$.

(ii) Linear trend $F=4{\cdot}48$, df$=1/70$, $P<0{\cdot}05$.

(iii) Regression equation (X = behaviour and Y = age) $X=0{\cdot}01\ K+0{\cdot}02$.

that the submissive behaviour toward other animals by females in transition fro infancy to juvenilehood differed significantly between age blocks (F 3·14, df 5/ $P<0{\cdot}05$). The rise and fall of observed frequencies depicted in figure 1 was tested trend analysis, which was characterized by a significant linear incremental trend 16·69, df 1/30, $P<0{\cdot}01$). Since the obtained equation was defined by a negati constant and a weak coefficient ($X=0{\cdot}01\ K-0{\cdot}01$), the submissive behaviour can predicted as gradually increasing from infancy to juvenilehood among fema (figure 1).

Submission by females also increased significantly when juveniles becar adolescents (F 2·71, df 5/30, $P<0{\cdot}05$). However, the observed frequencies chang their direction thrice which was significant as a cubic trend in statistical ter (F 8·87, df 1/30, $P<0{\cdot}01$). Observed frequencies plotted upon age blocks in figure shows that submissive behaviour decreased initially then increased till 5th a block, but returning to the initial level in the 6th block. The derived line regression equation in general predicted fast additions in submission as enteri into adolescence because the constant was positive and the regression coeffici was also substantial for each age block ($X=0{\cdot}02\ K+0{\cdot}02$).

The frequencies of aggression by others toward males in transition from juveni hood to subadult class significantly differed between age blocks (F 3·40, df 5/ $P<0{\cdot}01$). Age dependent changes in frequencies was best characterized by a line incremental trend (F 4·48, df 1/70, $P<0{\cdot}05$). Figure 3 shows that during juven period (i.e. in first 3 age blocks) the aggression received was less varied, howev during subadult stage males received more aggression. Such pattern was express by a linear regression equation in the form of gradual increase in aggress behaviours by other animals toward male subadults ($X=0{\cdot}01\ K+0{\cdot}02$).

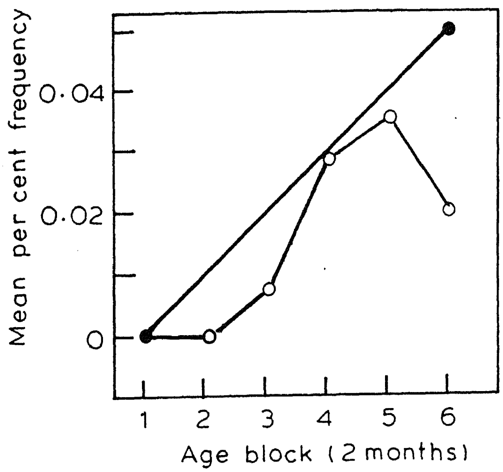

Figure 1. Mean per cent frequencies of submission to others by females in transition from infancy to juvenilehood. Straight line defines the best fit linear equation as X=0·01 K−0·01 (age from 6–18 months).

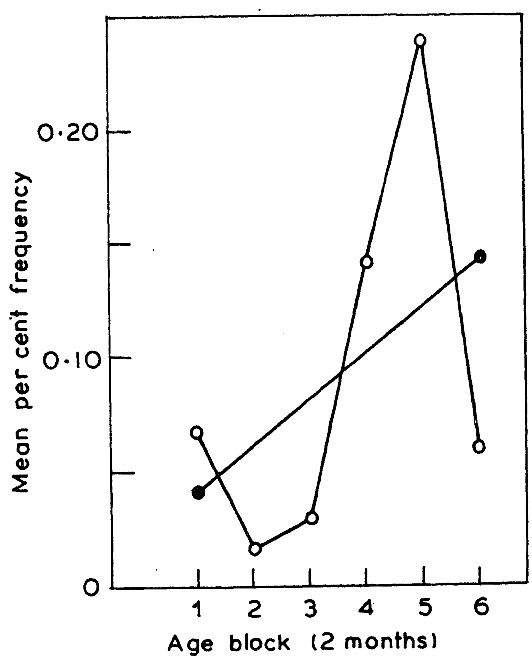

Figure 2. Mean per cent frequencies of submission to others by females in transition from juvenilehood to adolescence. Best fit linear function is by equation as X=0·02 K−0·02 (age from 18–30 months).

Kruskal-Wallis 'H' was applied to assess the significance between young, mature and old females (statistically parallel to chi-square distribution) aggressive and submissive behaviour (table 6). The obtained statistics were non-significant for both categories of aggressive behaviour, but significant for submissive behaviour by

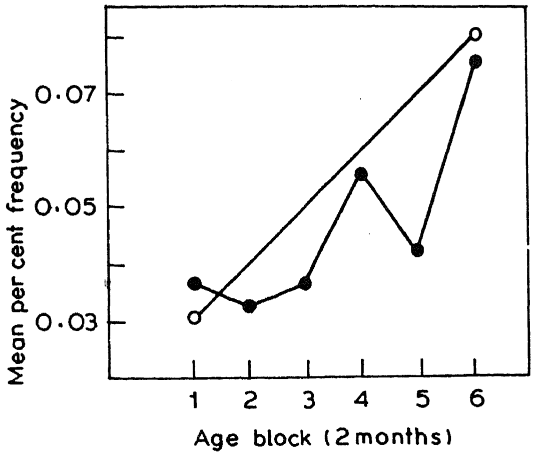

Figure 3. Mean per cent frequencies of aggression by others towards males in transition from juvenilehood to subadulthood. Linear equation for straight line was X=0·01 K+0·0 (age from 42–54 months).

Table 6. Mean per cent frequencies of young, mature and old females for 4 behavioural categories of agonistic behaviour and Kruskal-Wallis ANOVA for significance between them.

Behaviour category	Mean per cent frequencies Young	Mature	Old	χ^2 (df=2)	*P*
Aggression to others	0·07	0·06	0·05	2·69	NS
Aggression by others	0·07	0·03	0·03	1·23	NS
Submission to others	0·18	0·08	0·17	12·67	$<0·01$
Submission by others	0·11	0·08	0·02	6·79	$<0·05$

NS, Non-significant.

others (*H* 6·79, df 2, $P<0·05$). Mean per cent frequencies show that with increasin age females received less submission by others. Others submit to old females leas (mean=0·02%) and maximum to young female (mean=0·11). Mature female submit less to others (mean=0·08) than young (mean=0·18) and old female (mean=0·17).

Significant differences between young and mature adult males tested by Mann Whitney 'U' test (table 7). It showed that mature males were significantly higher i aggression to, aggression by and submission by others than the young adult stag In either stage, they rarely emitted submission toward other animals.

5. Discussion

Overall ontogenetic pattern of rhesus females was that submissive rather tha aggressive behaviour should emerge as a characteristic feature. Their submissio toward other animals slowly increased during transition from infancy to juvenile hood which further increased during way to adolescence. However, during matu adult years it decreased and again increased in old age. The pattern may b interpreted as a function of increased pressure from other animals in the core are

Table 7. Mean per cent frequencies of young and mature males for 3 behavioural categories and Mann-Whitney 'U' for difference between them.

Behaviour category	Mean % frequency Young	Mature	U	P
Aggression to others	0·05	0·07	3	<0·01
Aggression by others	0·08	0·21	0	<0·01
Submission by others	0·03	0·10	0	<0·01

of the group, as the females have to be integrated in the central space of the group. Similarly, females received less submission by other animals as they mature, because they themselves were less aggressive to others. Less submission during mature adulthood may be due to strong affiliation with males and support of kinship.

Aggressive behaviour among males increased linearly when they become sub adult from juveniles because juvenilehood among rhesus males is generally peripheral and solitary, whereas sub-adult males start taking interest in group activities to secure a place in group space. Interestingly, mature males received and emitted more aggression than young adults; this may have been due to the role of mature males in intragroup strifes. Typically, as males advance in age, the submissive behavioural elements are dropped from their behavioural repertoire. It seems that apart from other factors, the most important factor to influence the quantitative expression of aggressive and submissive behaviour may be age dependent spatial position of the animal in the group space, i.e. a consistent and stable dispersion of group members in an area. Assuming central position in the group is a motivating force for animals which enhances the likelihood of aggressive and submissive behaviours. Moreover, these patterns are not only susceptible to changes during growing years but also during adulthood. Old animals become more submissive and display a general disengagement in group activities, as reported by Hauser and Tyrrell (1984).

By and large, the observation support the findings of Altmann (1968) reporting that the agonistic behaviour of adult males is somewhat more likely to be aggressive than that of adult females, while the behaviour of adult females is more likely to be submissive.

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Morphogenesis of the foetal membranes and placentation in the Indian molossid bat, *Chaerephon plicata* (Buchanan)

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Abstract. In *Chaerephon plicata* the definitive amnion is formed by the development of folds. The yolk-sac splanchnopleure undergoes progressive collapse and folding until it ultimately remains as a free gland-like structure with hypertrophied endodermal and mesodermal cells. It acts as an active haemophagous organ after limb-bud stage. An extensive chorio-vitelline placenta, which is formed during early gestation is progressively abolished and replaced by the chorio-allantoic placenta. Two kinds of chorio-allantoic placenta are formed—a diffuse endotheliochorial placenta, which persists until about mid-pregnancy, and a definitive discoid placenta which is mesometrically located, labyrinthine and haemomonochorial.

Keywords. Development; foetal membranes; placentation; bat; Molossidae.

1. Introduction

The family Molossidae includes over 80 species (Koopman 1984; Hill and Smith 1985) and representatives of this family are found throughout the world except in the very cold regions and in the Sahara desert. Yet some details of development and the structure of the foetal membranes and placenta are known of only one new world species, *Tadarida brasiliensis cynocephala* (Mossman 1937; Stephens 1962, 1969; Stephens and Easterbrook 1968, 1969, 1971; Stephens and Cabral 1971). These studies have revealed that the yolk-sac splanchnopleure in this species becomes free, undergoes collapse and the endodermal cells hypertrophy. Thus, the yolk sac acquires a gland-like appearance during the final stages of gestation. The endodermal cells of the yolk sac store glycogen and protein until advanced pregnancy. An extensive chorio-vitelline placenta is present until the limb-bud stage of development of the foetus after which it is replaced by chorio-allantoic placenta. Two kinds of chorio-allantoic placenta develop in this bat—a transient, diffuse, labyrinthine endotheliochorial placenta, which occur until mid-pregnancy, and a definitive, discoid, labyrinthine, haemochorial placenta on the mesometrial aspect of the uterus.

The present paper embodies descriptions of the morphogenesis of the foetal membranes and placenta of *Chaerephon plicata*. Apart from the fact that this is the first report on the embryology of an Indian molossid bat, this study has revealed for the first time the manner by which the final placenta comes to develop on the abembryonic side—an unique feature unmatched by any species of any other family of bats. Secondly, the yolk sac in this species acts as an haemophagous organ after mid-pregnancy. This also is a feature unknown in any other bat. The early embryology of this species was described in a previous paper (Pendharkar and Gopalakrishna 1984).

2. Materials and methods

The specimens were collected from old buildings at Khandwa, Madhya Pradesh, India at frequent intervals from the beginning of July to the end of September during 3 successive years—1977, 1978, 1979. The specimens were either shot down with a small calibre air gun or captured with butterfly nets. Altogether 128 pregnant specimens at different stages of pregnancy were examined. The genitalia of the specimens were fixed in various fixatives, such as neutral formalin, Rossman's, Baker's and Carnoy's fixative. The tissues were dehydrated by passing through graded ethanol, cleared in xylol, embedded in paraffin and serially sectioned at 6 to 7 μ thickness. Most of the sections were stained with Ehrlich's or Harris' haematoxylin and counterstained with eosin, cleared in xylol and mounted in DPX. A few sections from each series were stained by the periodic acid-Schiff (PAS) procedure (Pearse 1968) followed by staining with Weigert's haematoxylin and some by Mallory triple procedure.

3. Results

3.1 *Neural groove stage*

Six specimens with embryos at different stages of development of the neural groove were available for this study. Figures 1 and 7 illustrate sections of the uterus containing an embryo at an advanced neural groove stage of development. The embryonic plate with the neural groove in the center was facing the antimesometrial side of the uterus. In an earlier report (Pendharkar and Gopalakrishna 1984) it was shown that the expansion of the embryonic plate of the implanted trilaminar blastocyst had resulted in stretching and final tearing away of the trophoblastic layer overlying the embryonic plate resulting in exposing the embryonic plate to the potential uterine lumen. This situation continued in the neural groove stage, when the embryonic plate faced the uterine endometrial tissue since the trophoblastic layer and the uterine epithelium were absent from this region. Folds of definitive amnion had formed at the margins of the embryonic plate and had grown for a short distance towards the dorsal side of the embryonic disc. Exocoelom had formed at the margins of the embryonic disc and had extended into the amniotic folds and to a little distance on the lateral sides of the yolk-sac wall. An extensive chorio-vitelline placenta was present in relation to the lateral and mesometrial sides of the uterus. A zone of syncytiotrophoblast occupies about a fourth of the thickness of the uterine wall on the lateral sides of the uterus and numerous short, solid, conical or bulbous projections of the basal cytotrophoblastic layer had entered the syncytiotrophoblastic zone in many places along the foetal margin of the placenta (figure 8). On the abembryonic side adjacent to the tubo-uterine junction, and towards the mesometrial aspect of the uterus, the trophoblast had undergone proliferation and had formed a thick pad (figure 9). On all the sides of the implantation chamber the endometrial tissue immediately overlying this trophoblastic zone had formed a mantle of symplasma caused by the merging of disintegrated terminal segments of the uterine glands and cells of the mesometral stroma, both components losing cell membranes (figures 10, 11). The endometrial

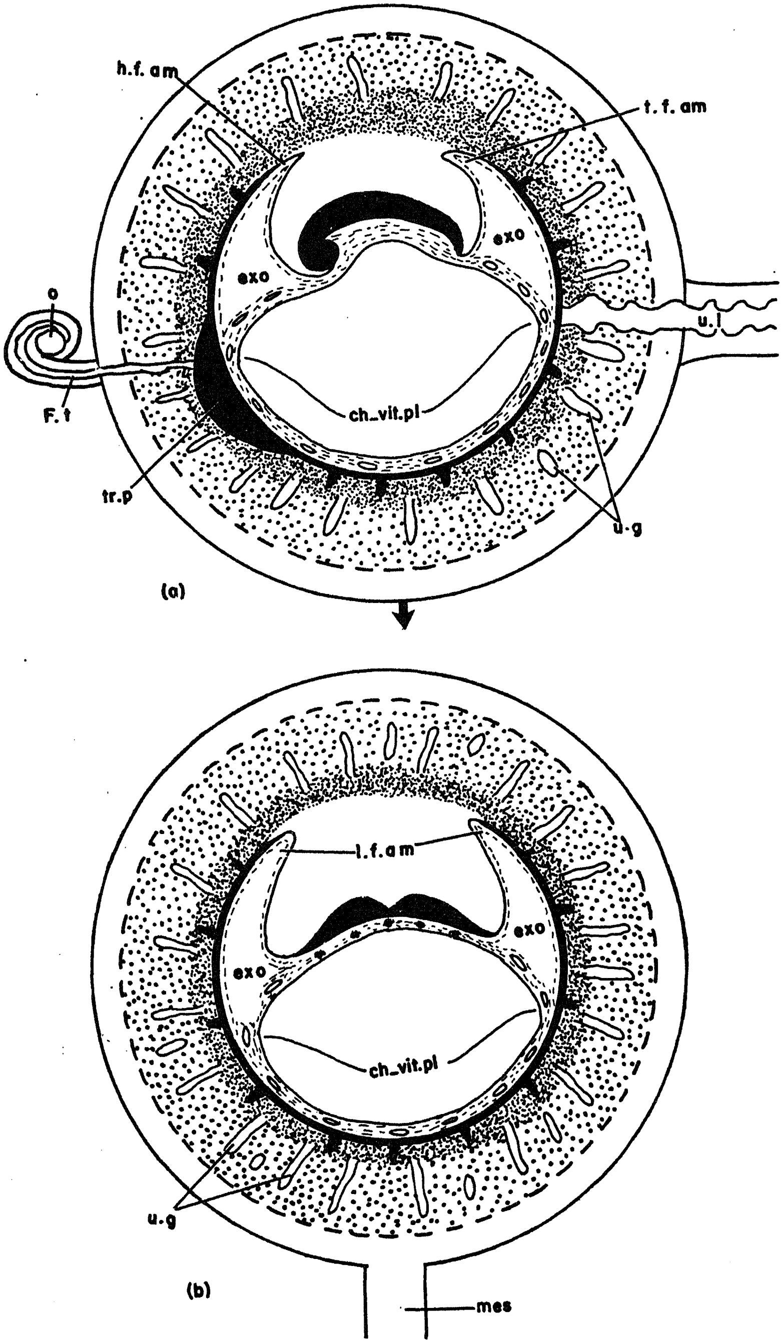


Figure 1. Sagittal (**a**) and transverse (**b**) section of the uterus containing an embryo in the later neural groove stage of development.

tissue in the deeper regions was loose and had numerous fluid-filled intercellular spaces. A thin zone of compact endometrium occurred between this zone and the myometrium.

3.2 *Stage of early allantoic attachment*

The following descriptions are based on the examination of 4 progressively advanced pregnant uteri each containing a foetus in which the allantois had grown across the exocoelom and had established contact with the uterine wall on the antimesometrial and a part of the lateral sides (figures 12, 13). The allantois was composed of a mass of mesenchyme surrounding an endodermal allantoic duct, which had expanded distally into a vesicle. Numerous foetal capillaries were present in the allantoic mesenchyme. As a result of the extension of the exocoelom into the yolk-sac wall the embryonic segment of the yolk-sac splanchnopleure had become separated from the chorion, but it retained contact with the endometrium on the mesometrial and a part of the lateral sides of the uterus. Thus, there was a chorio-allantoic placenta on the antimesometrial and a part of the lateral sides of the uterus and a chorio-vitelline placenta on the rest of the regions of the uterus.

The histological changes consisted of invasion of the syncytiotrophoblastic zone by the basal cytotrophoblastic layer in the form of solid or hollow projections (figures 14, 15). Maternal blood capillaries in this zone had distinct endothelial lining composed of hypertrophied endothelial cells (figure 16). Some of the maternal capillaries in the zone of symplasma had broken down forming pools of extravasated blood (figure 17) and this was ingested by syncytiotrophoblast. The cytotrophoblastic pad, which was noticed adjacent to the tubo-uterine junction in the previous stage, had become thicker and had expanded towards the mesometrial side.

3.3 *Early limb-bud stage*

This stage is characterized by the foetus possessing one pair of limb-buds, the presence of the chorio-vitelline placenta over a small area of the gestation sac and the occurrence of an extensive chorio-allantoic placenta in the rest of the uterus. Figures 2 and 18 illustrate the general arrangement of the foetal membranes at this stage. The allantois with the allantoic vesicle had expanded considerably and was applied to a wide area of the placenta on the antimesometrial and lateral sides, and had grown cranially up to the region of the trophoblastic pad mentioned earlier. Consequently, the yolk-sac splanchnopleure had become separated from the chorion resulting in the abolition of the chorio-vitelline placenta from these regions. Only a small distal abembryonic area of the yolk-sac splanchnopleure retained its contact with a part of the mesometrial and a part of the lateral side of the gestation sac. In the freed region the yolk-sac splanchnopleure was thrown into folds (figure 19) and the endodermal cells had hypertrophied and had become cuboidal and contained vesicular nuclei. The mesodermal cells of the yolk-sac wall were flat.

The placenta had undergone marked histological changes. It was in the form of a spherical shell of syncytiotrophoblast occupying about a third of the thickness of the uterine wall at the level of gestation on all the sides except in the region of the cytotrophoblastic pad. The basal cytotrophoblastic layer had penetrated deep into the syncytiotrophoblastic zone in the form of deep hollow inpushings (figure 20) some of which had become branched and inter-connected. Extraembryonic mesenchyme had entered these hollows. Whereas in the regions of the chorio-allantoic placenta the foetal blood capillaries had entered the placental complex, in

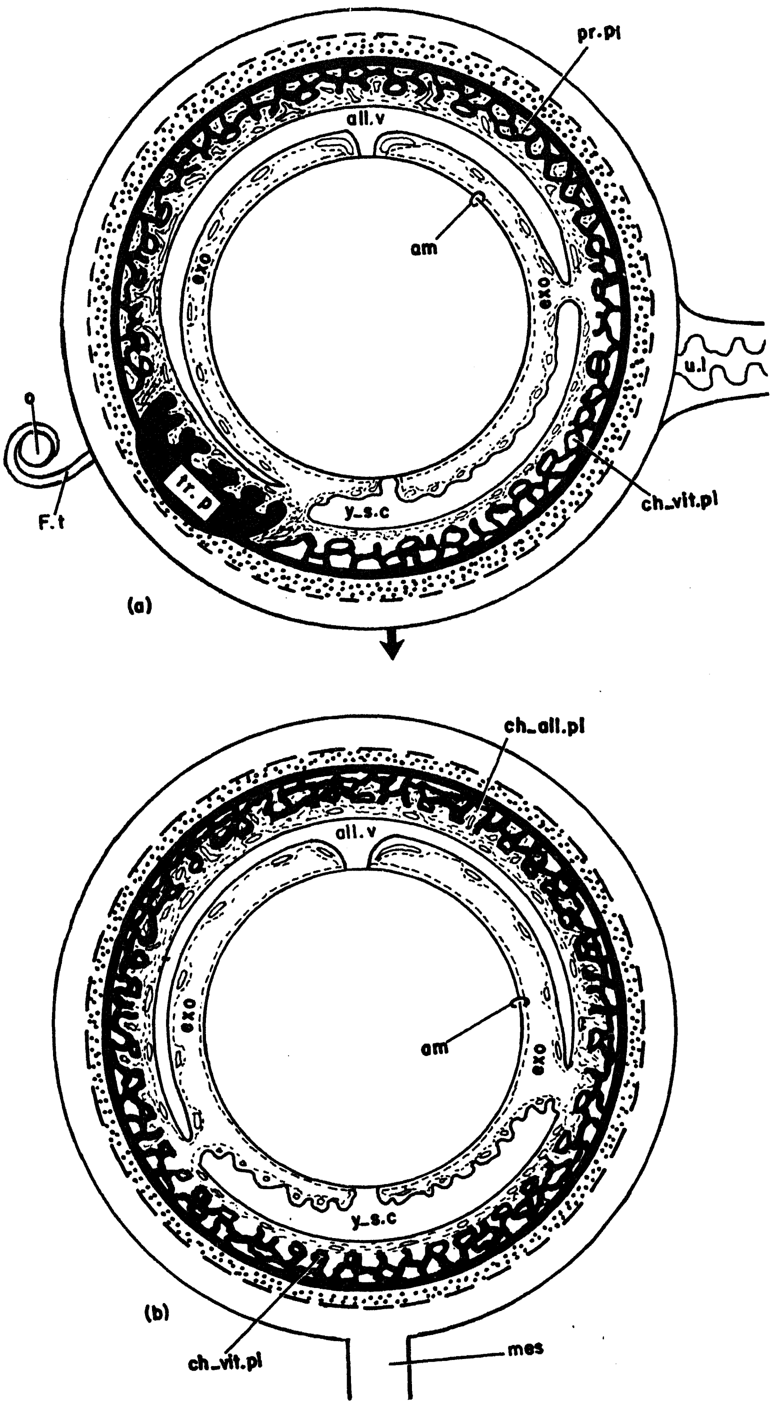


Figure 2. Sagittal (**a**) and transverse (**b**) section of the uterus showing arrangement of the foetal membranes in early limb-bud stage.

the region of the chorio-vitelline placenta the vitelline vessels did not enter the placenta complex, but lay on the foetal border of the placenta. Maternal blood capillaries in the placenta had distinct endothelial lining with slightly hypertrophied endothelial cells in most places (figure 21). The endometrial symplasma consisted of large masses of cytoplasm containing widely scattered nuclei, and many pools of extravasated maternal blood occurred in this zone.

The trophoblastic pad mentioned earlier had become thick and had occupied a wide area on the mesometrial side of the uterus near the tubo-uterine junction. The foetal surface of the pad had numerous pits which gave the trophoblastic pad an indented appearance in sectional views. Allantoic mesenchyme and foetal capillaries had occupied these pits. Several maternal blood capillaries pierced the pad from the uterine side and they had flat endothelial cells for about half their length within the pad and were devoid of endothelial lining in the rest of the regions.

3.4 *Late limb-bud stage*

This stage is characterised by the presence of two pairs of limb-buds in the foetus and the complete separation of the yolk-sac splanchnopleure from the chorion on all the sides with the consequent complete abolition of the chorio-vitelline placenta. Figures 3 and 23 illustrate the gross morphology of the foetal membranes at this stage of gestation. The cavity of the yolk sac had become reduced in its extent since the separated splanchnopleure was pushed centripetally by the expanding amnion and the extension of exocoelom on all its sides. In many places the free vascular yolk-sac splanchnopleure had been thrown into folds in which the endodermal cells had hypertrophied and had become columnar and crowded together. The mesodermal cells were still flat in most places, but were slightly enlarged in the regions of the folds. Vitelline vessels were present on all the sides of the yolk sac.

Allantois carrying foetal capillaries had spread over the entire foetal surface of the gestation sac thereby converting the entire placenta into the chorio-allantoic type. The allantoic vesicle was considerably reduced and occurred as narrow spaces in the allantoic mesenchyme. The allantoic duct was, however, present. The placenta could be recognised into two moieties with different histological characters—a diffuse placenta and a mesometrially located discoid placenta. In the diffuse placenta cytotrophoblastic villi carrying foetal mesenchyme and blood capillaries had invaded deep into the syncytiotrophoblastic shell. Most of the villi were branched and the branches of adjacent villi had fused in the deeper regions (figure 24). Consequently, the syncytiotrophoblast, in which maternal blood capillaries lay, had become compressed into tubules, the central lumen of each tubule being the persistent maternal blood capillary, which, in most places, had hypertrophied endothelial cells (figure 25), and the wall of the tubules being made up of an inner layer of syncytiotrophoblast and an outer layer cytotrophoblast. The diffuse placenta was, therefore, labyrinthine and endotheliochorial. Numerous large multinucleate endometrial cells were present on the maternal border of the placenta (figure 24). A thin layer of loose endometrial tissue occurred between this zone and the myometrium.

The discoid mesometrial moiety of the placenta was in the form of a lobulated (figure 26) thick disc embedded in the uterine wall with the foetal surface protruding slightly into the exocoelom. A mass of allantoic mesenchyme distinctly separated

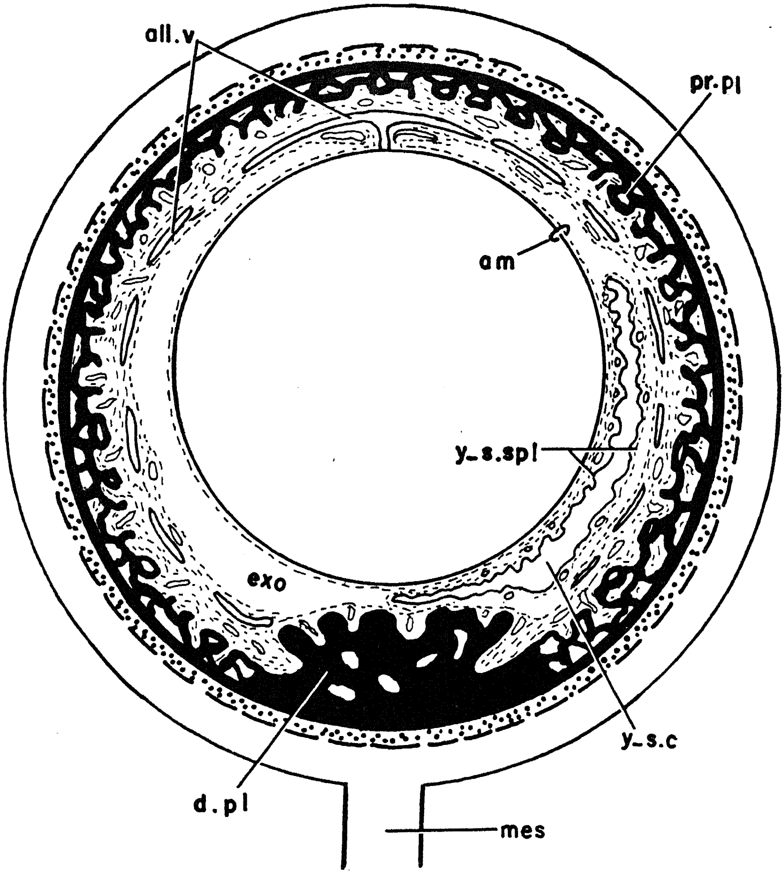


Figure 3. Arrangement of the foetal membranes at late limb-bud stage.

the discoid placenta from the diffuse placenta. The placental disc was composed of several lobes separated from one another by foetal mesenchyme carrying foetal blood vessels. Each lobe was made up of a labyrinth of loosely arranged placental tubules the core of which had a maternal blood space without endothelial lining. In the deeper regions of the disc, i.e. adjacent to the maternal endometrial tissue, some of the maternal blood capillaries had endothelial lining composed of flat cells resting on a thin basement membrane. The wall of the tubules was made up of a row of cytotrophoblastic cells. There was a thin layer of endometrial tissue with compactly arranged cells on the maternal border of the placental disc, and this region was pierced by maternal blood capillaries which ultimately extended into the placental tubules. Outside this zone the endometrium was loose and spongy, and this was contiguous with the loose endometrial tissue in the rest of the uterine wall around the diffuse placental zone.

3.5 *Mid-pregnancy*

The general arrangement of the foetal membranes at this stage of gestation is

illustrated by figures 4 and 27. The free yolk sac had undergone further collapse and lay in the exocoelom partly towards the lateral and partly towards the mesometrial sides. The yolk-sac splanchnopleure was thrown into numerous folds and the cells of the endodermal layer had undergone marked hypertrophy. The mesodermal cells had also enlarged and had formed a covering of cuboidal cells on the exocoelomic surface of the yolk-sac splanchnopleure. In many places the mesodermal layer occurred in the form of numerous blunt outpushings projecting into the exocoelom (figure 28). The entire yolk-sac wall was richly vascularised. A large amount of extravasated maternal blood was present around the yolk sac and the mesodermal cells of the yolk-sac splanchnopleure were engorged with ingested maternal erythrocytes.

The placenta occurred on all the sides of the gestation sac, and while the diffuse placenta was thinner than in the previous stage, the discoid placenta had become more extensive and thicker. Enlarged endothelial cells lay on a thick basement membrane in the maternal blood capillaries in the placenta (figure 29). There was no remnant of the endodermal allantoic duct or the vesicle, but the allantoic mesenchyme carrying foetal blood vessels had spread over the entire foetal surface of the placenta.

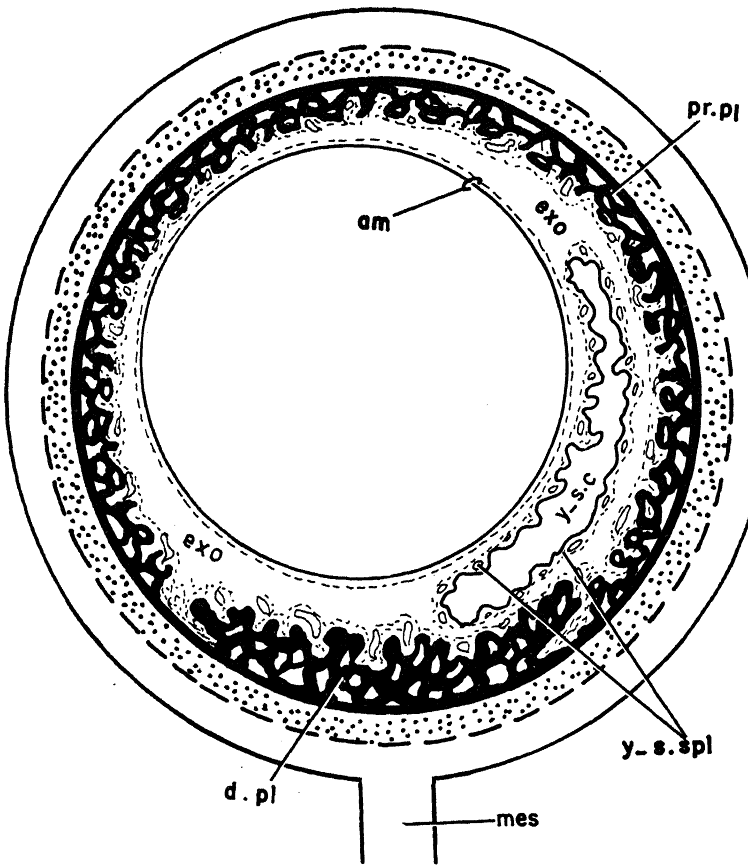


Figure 4. Arrangement of the foetal membranes at mid-pregnancy.

The definitive placental disc on the mesometrial side was in the form of a wide thick bulb whose narrower base was embedded deep in the uterine wall and the broader end protruded into the exocoelom. There was deep cleft in the center of the placenta which separated the placental disc into nearly equal halves. Each half of the placenta was composed of a network of a large number of placental tubules inside which circulated maternal blood. There was no remnant of the maternal endothelium in the placental tubules—each tubule being composed of a single layer of cytotrophoblast. Allantoic mesenchyme and foetal capillaries occupied the inter-tubular areas. On the maternal border of the placenta occurred a continuous layer of trophoblast and this continued as the trophoblastic component of the chorion in the primary placenta. There was no change in the histology of the rest of the uterine endometrium.

A little after mid-pregnancy (figure 5) the diffuse primary placenta had disappeared altogether and the discoid definitive placenta had become thicker. The yolk sac had undergone further collapse and lay mostly on the lateral side of the gestation sac. The histological structure of the yolk-sac splanchnopleure and the placenta remained nearly the same as before.

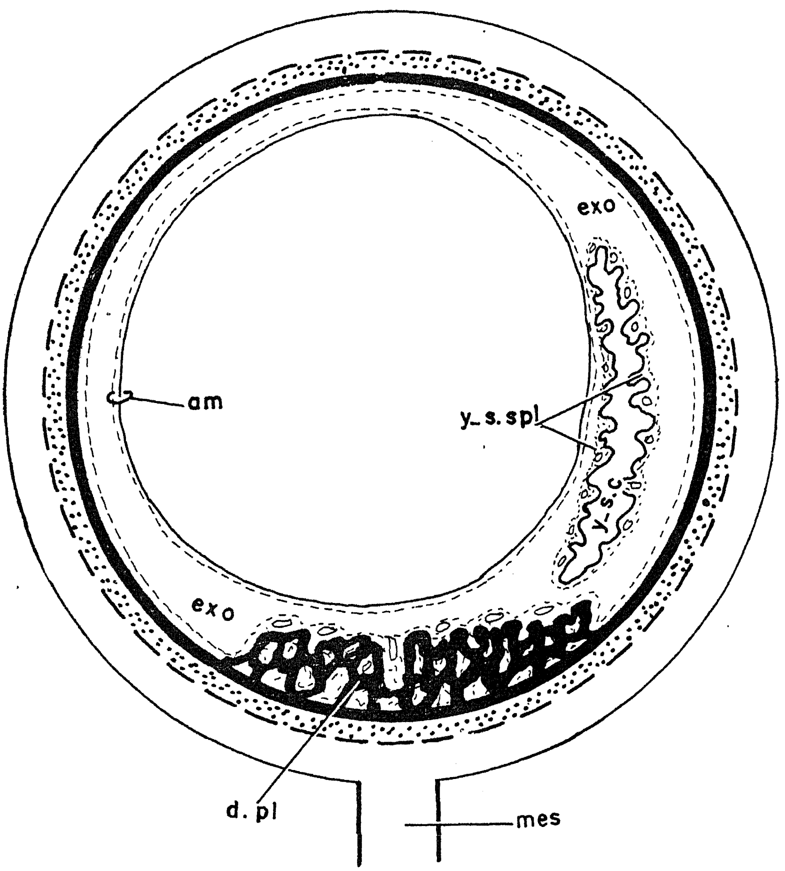


Figure 5. Arrangement of the foetal membranes at advanced pregnancy.

3.6 *Full term*

The general arrangement of the foetal membranes at full term is illustrated in figures 6 and 30. The enormous enlargement of the foetus had resulted in the stretching of the uterine wall, which had consequently become thin on all but the mesometrial side, where the discoid chorio-allantoic placenta was present.

The yolk sac had undergone nearly complete collapse resulting in the approximation of the walls of the yolk sac and the abolition of the yolk-sac lumen from most places (figures 31, 32). The lumen, where present, occurred as small spaces bounded by hypertrophied columnar endodermal cells. The mesodermal layer composed of hypertrophied polygonal vacuolated cells which occurred in the form of numerous blunt villi which projected into the exocoelom. These histological changes gave the yolk sac a gland-like appearance. A large quantity of extravasated maternal blood was present in the exocoelom and the yolk-sac was bathed in this

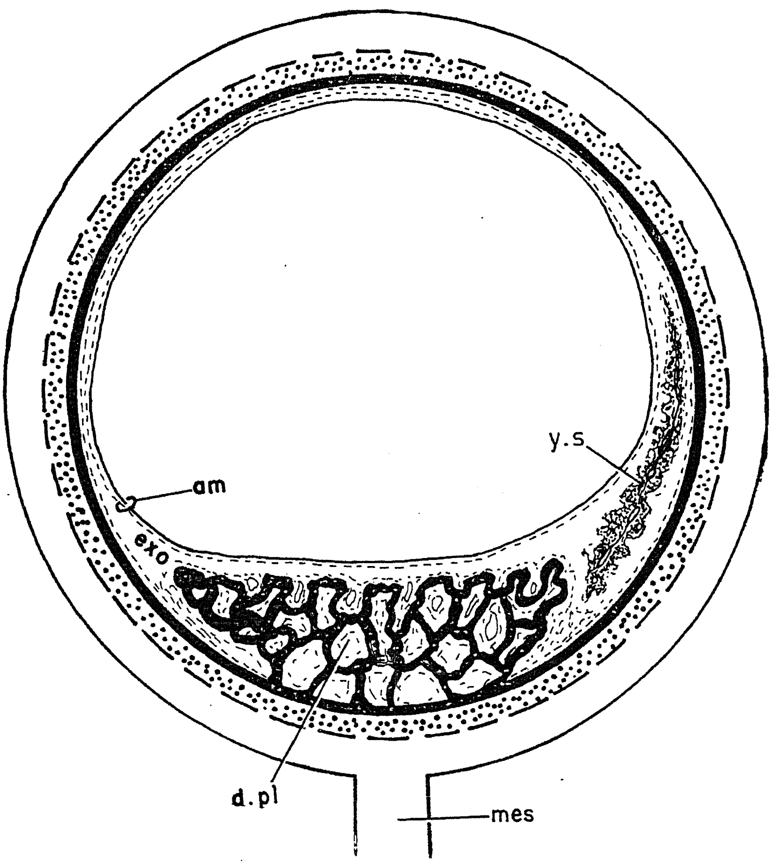
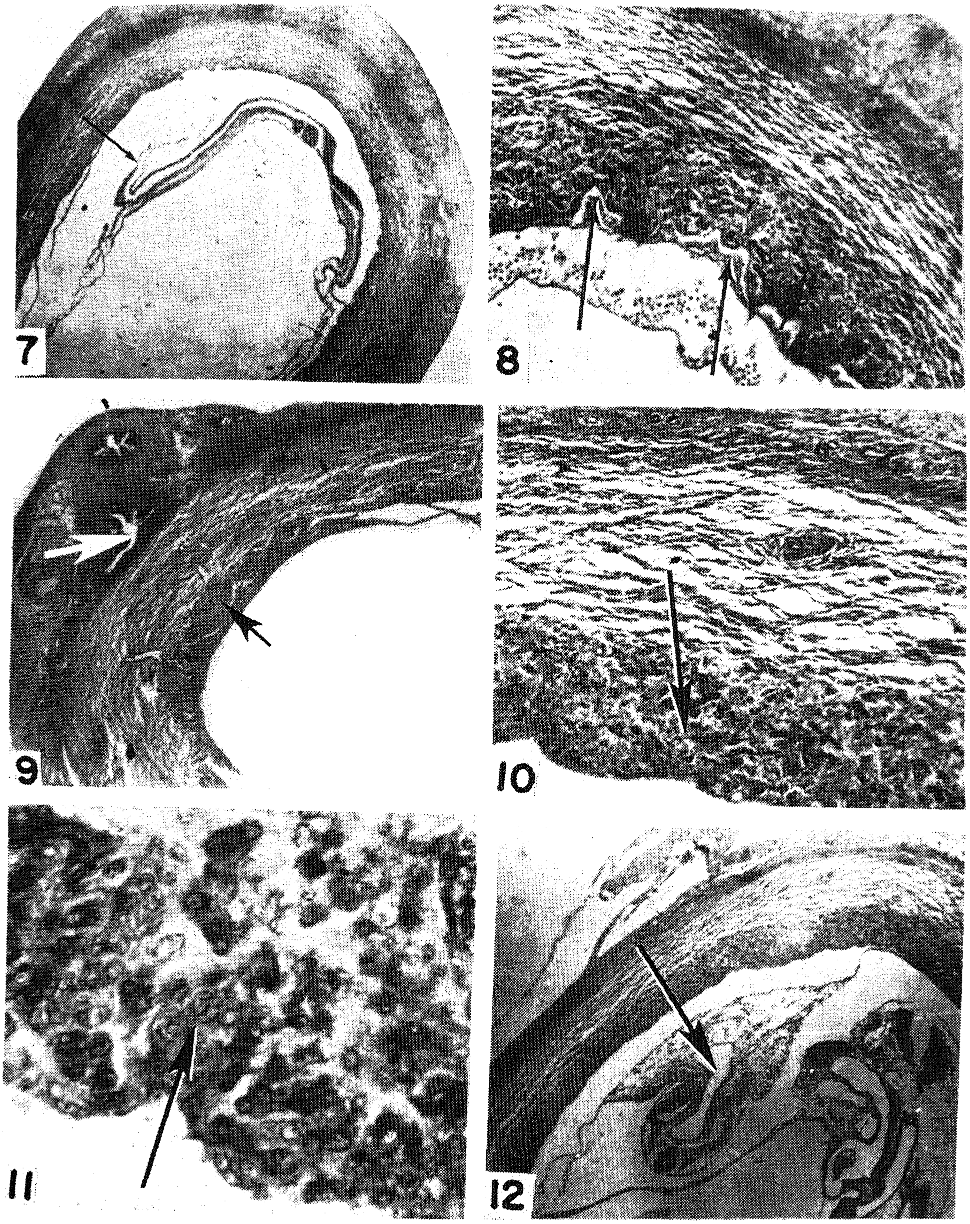


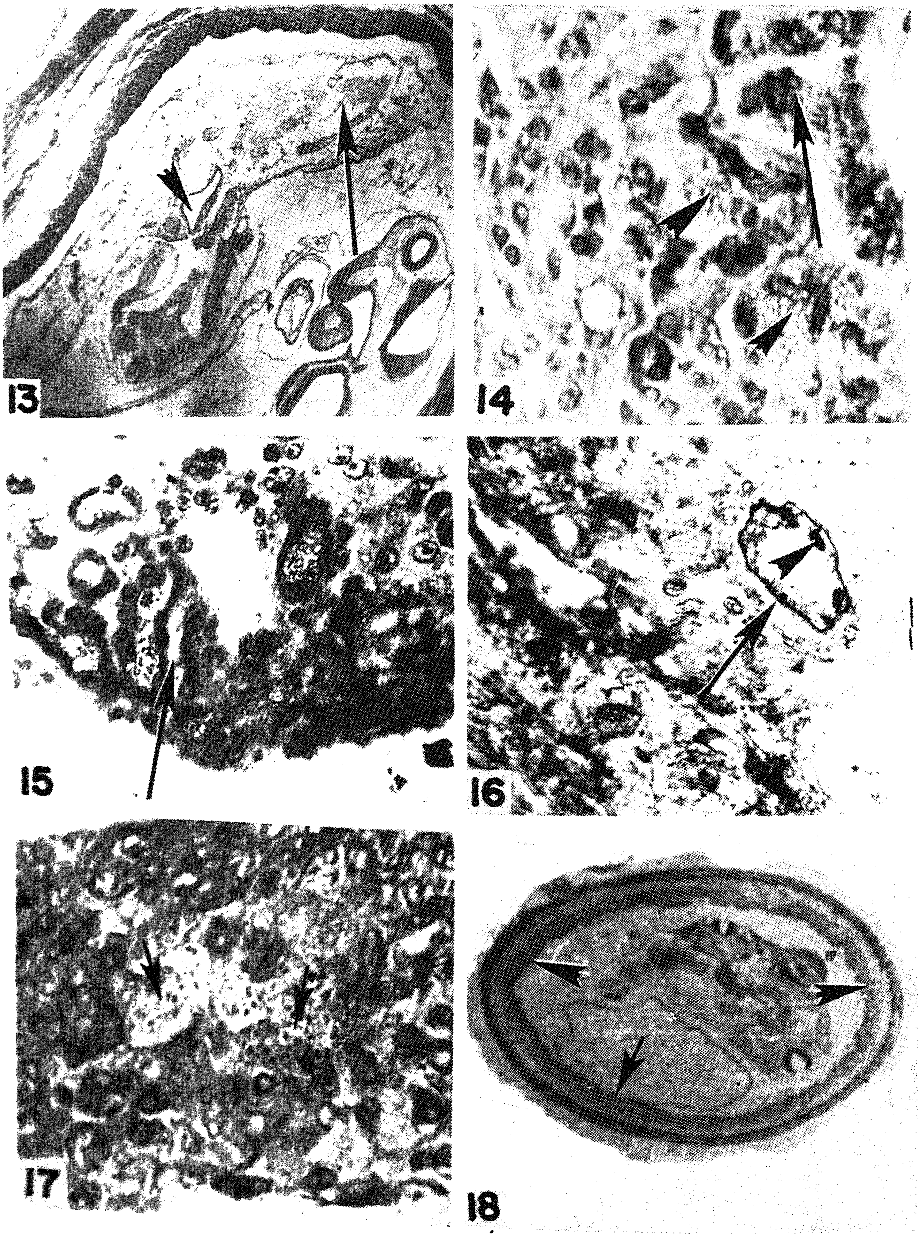
Figure 6. Arrangement of the foetal membranes at full term.
(Abbreviations used in figures 1–6: all.v, Allontoic vesicle; am, amnion; ch-all.pl, chorio-allontoic placenta; d.pl, definitive placental disc; exo, exocoelom; F.t, fallopian tube; h.f.am, head fold of amnion; l.f.am, lateral fold of amnion; mes, mesometrium; o, ovary; pr.pl, primary (diffuse) placenta; t.f.am, tail fold of amnion; tr.p, trophoblastic pad; u.g, uterine gland; u.l, uterine lumen; y.s, yolk sac; y-s.c, yolk-sac cavity; y-s.pl, yolk-sac splanchnopleure).



Figures 7–12. **7.** Part of the transverse section of the uterus containing an embryo in advanced neural groove stage of development. Arrow points to a lateral amniotic fold (× 24). **8.** Part of the uterine wall at neural groove stage. Note projections of the basal trophoblastic layer (long arrows) into the zone of syncytiotrophoblast (short thick arrow), (× 80). **9.** The abembryonic part of the section of the uterus containing an embryo in neural groove stage of development. Black arrow points to the trophoblastic pad adjacent to the tubo-uterine junction (white arrow) (× 52). **10.** Part of the uterine wall of the uterus at neural groove stage. Note the layer of syncytiotrophoblast (arrow) and a deeper layer of loose endometrium (× 92). **11.** Enlarged part of figure 10. Note a bulbous intrusion of trophoblastic layer (arrow) into the zone of syncytiotrophoblast (× 260). **12.** Part of the section of the uterus containing an embryo with early allantoic attachment. Note the allantoic vesicle (arrow) within a large mass of mesenchyme (× 10).

blood. The mesodermal layer was in the form of numerous blunt projections protruding into the exocoelom, and the cells had undergone enormous hypertrophy and were engorged with maternal erythrocytes from the extravasated blood.

The definitive chorio-allantoic placental disc was deeply undercut by the paraplacental chorionic layer on all the sides so that in sectional views the placenta appeared in the form of a thick bulb on the mesometrial side. It was composed of



Figures 13–18. For caption, see p. 165.

numerous lobules separated by strands of allantoic mesenchyme. Each lobule was composed of numerous highly branched and anastomosed placental tubules (figure 33) whose walls were made up of cytotrophoblast and in whose lumen circulated maternal blood (figure 34). Thus, a 3 dimensional network of placental tubules had formed with the meshes of the net being occupied by foetal mesenchyme and foetal blood capillaries. In many places fine allantoic capillaries appear to be embedded in the wall of the placental tubules (figure 35), and the trophoblast in these places had become so much attenuated that only a thin strand of cytoplasm formed the interhaemal membrane separating foetal and maternal bloods. The definitive chorio-allantoic placenta of *Chaerephon plicata* can, therefore, be described as mesometrial, discoidal, labyrinthine and haemomonochorial with only the cytotrophoblast being present.

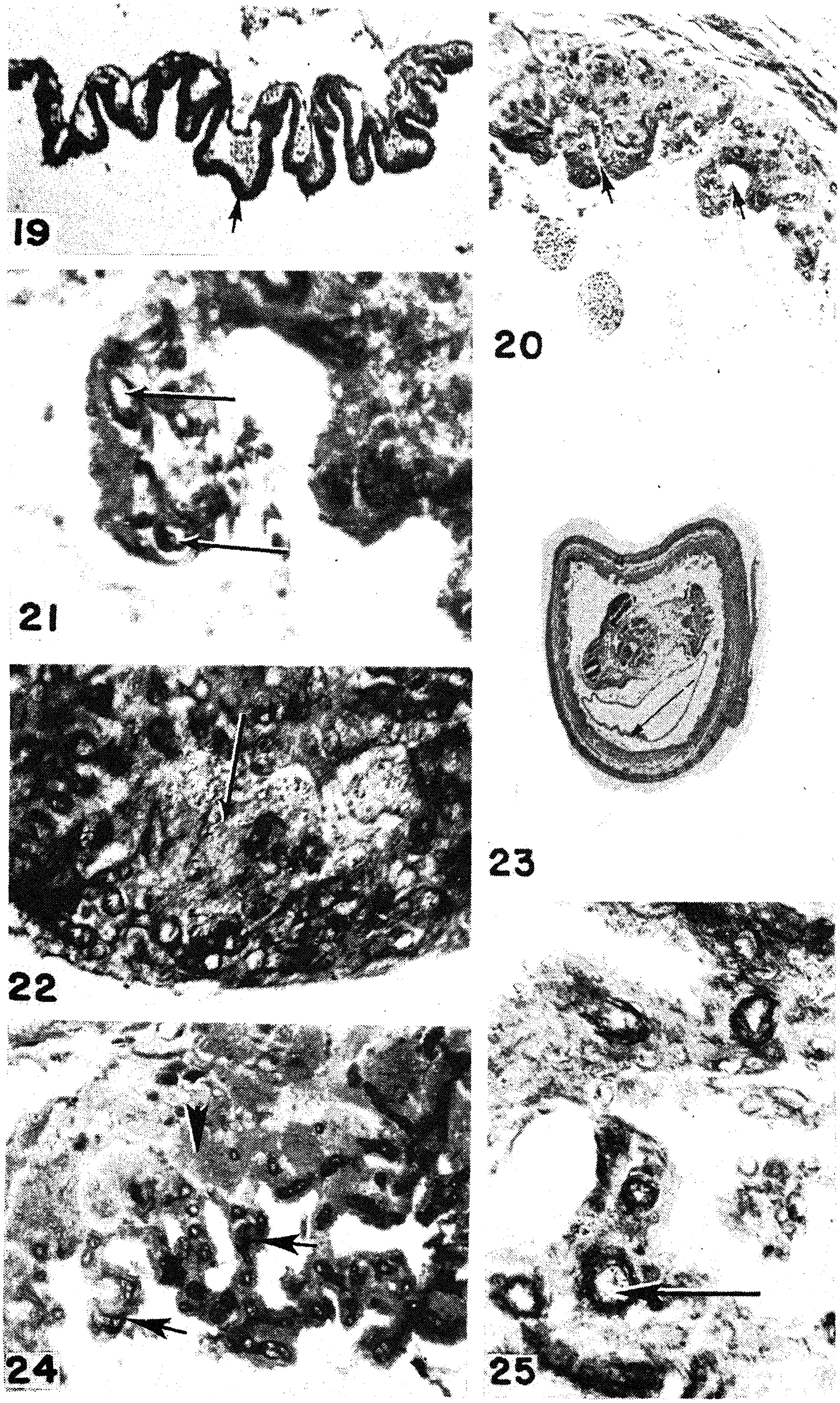
A cytotrophoblast shell, 3 to 4 cells thick, occurred on the uterine side of the placental disc (figure 32), and this was contiguous with the trophoblastic layer of the paraplacental chorion. The utero-placental junctional zone had a thin strip of endometrium in which there were many binucleate and multinucleate giant cells (figure 36). A zone of loose tissue with numerous fluid-filled spaces occurred between this zone and the myometrium. Evidently, this was the zone of separation of the parturient.

The umbilical cord (figure 37) carried two allantoic arteries, an allantoic vein, a vitelline artery and a vitelline vein. All these blood vessels were embedded in a thick mass of mesenchyme.

4. Discussion

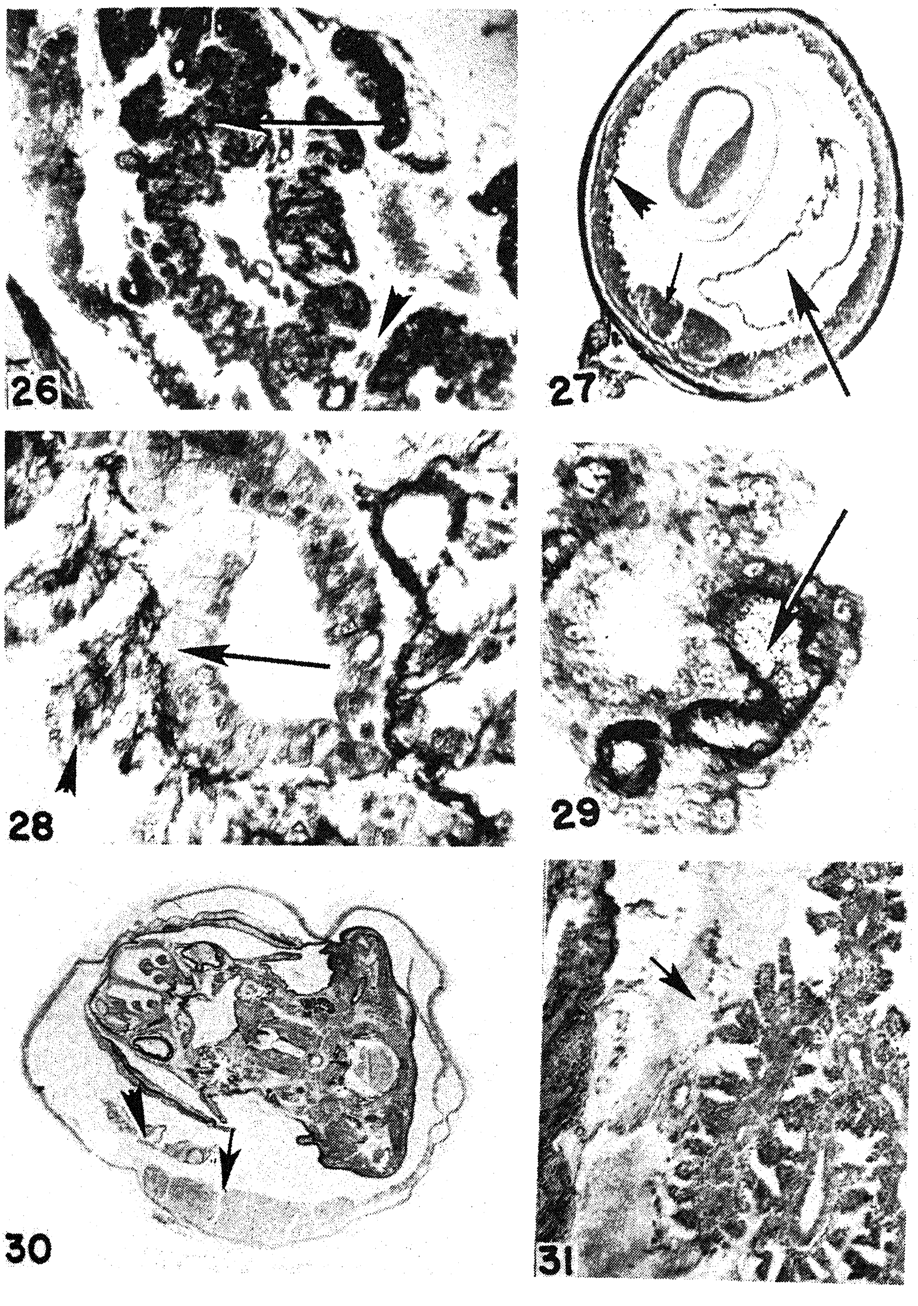
The structural changes undergone by the yolk sac during the development of *Chaerephon plicata* resemble those of *Tadarida brasiliensis cynocephala* (Stephens 1962, 1969), but with some important differences. After mid-gestation the free yolk sac is bathed in extravasated maternal blood, and maternal erythrocytes are directly ingested by hypertrophied mesodermal cells of the collapsed yolk-sac splanchnopleure. Evidently, during advanced stages of gestation of *Chaerephon plicata* the yolk sac is an active haemophagous organ and is an important avenue for the transport of iron and proteins from mother to the foetus. This unique feature of the cells of the yolk sac is noticed for the first time in any bat. Syncytiotrophoblast adjoining maternal symplasma also ingests maternal blood from the pools formed by the break down of maternal blood capillaries in these regions. This feature was noticed by Stephens (1962) in *Tadarida brasiliensis cynocephala*. Evidently, this seems to be an important method of transport of iron and proteins from the mother to the foetus during early gestation of molossid bats. The only other structure, where maternal blood is directly ingested by embryonic tissue in bats is the placental haematoma in some emballonurid bats (Gopalakrishna 1958; Wimsatt and Gopalakrishna 1958).

The second unique feature of the embryology of molossid bats is the formation of a definitive placental disc on the abembryonic aspect in relation to the earlier stages of development. In all other bats the allantoic placental disc is formed in that part of the uterus towards which the embryonic disc is oriented during early development. This is because the abembryonic region is occupied by the yolk sac and the chorio-vitelline placenta. Hence, the allantois grows towards the dorsal side

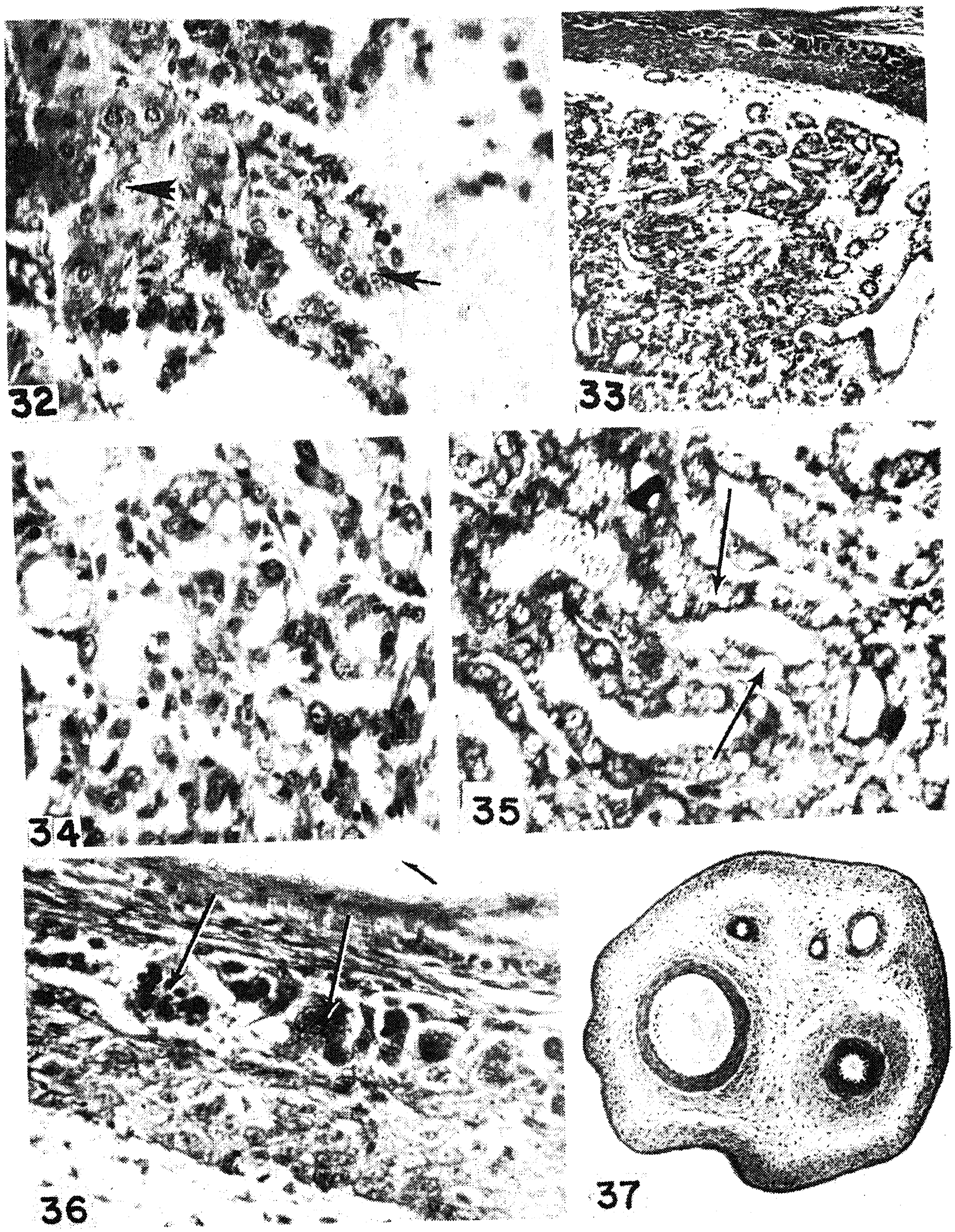


Figures 19–25. For caption, see p. 166.

of the embryonic plate—i.e. towards the side opposite to the one where the yolk sac occurs—and establishes contact with the uterine wall and forms the definitive allantoic placenta. In molossid bats, although initially the allantois grows towards the dorsal side of the embryonic plate as in all other bats, it rapidly expands towards the abembryonic regions and progressively replaces the chorio-vitelline



Figures 26–31. For caption, see p. 166.



Figures 32–37. **32.** Part of the yolk sac at full term. Arrow points to hypertrophied mesodermal cells bathed in extravasated maternal blood. Arrowhead points to endodermal cells (× 260). **33.** Part of the definitive placenta at full term (× 80). **34.** Part of the placental labyrinth. Note the occurrence of a single layer of trophoblast in the walls of the tubules (× 220). **35.** A few placental tubules at full term. Arrows point to fine foetal capillaries embedded in the wall of the placental tubule (× 280). **36.** Part of the utero-placental junction at full term. Arrows point to multinucleate endometrial giant cells (× 160). **37.** Section of the umbilical cord at full term (× 50).

placenta until it completely encompasses the yolk sac and establishes contact with a predetermined site on the mesometrial side—the side opposite the one towards which the embryonic disc was oriented during earlier stages. Although Stephens (1962, 1969) noticed the occurrence of definitive placenta on mesometrial side while the embryonic disc during early stages was oriented antimesometrically in *Tadarida brasiliensis cynocephala* (another molossid bat), he did not describe the unique manner in which this had been accomplished in this bat. It is, therefore, evident that the development of the allantois in Molossidae is unique and the definitive placenta becomes mesometrial secondarily.

The development of the allantoic placenta in two stages is yet another characteristic feature of molossid bats. There is a diffuse, labyrinthine, endotheliochorial placenta, which becomes progressively reduced until it disappears altogether a little after mid-gestation leaving only a discoid, mesometrially located, labyrinthine haemomonochorial placenta during the final stages of pregnancy.

Acknowledgement

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Figures 13–18. **13.** Part of the section of the uterus at advanced stage of allantoic attachment. Note the mass of allantoic mesenchyme (long arrow) containing a part of the allantoic vesicle (arrowhead) (× 14). **14.** Part of the uterine wall at the stage of early

allantoic attachment. The arrow points to a solid cord of trophoblast which had entered the syncytiotrophoblastic zone. Arrowheads point to pools of maternal blood in the zone of symplasma (× 260). **15.** Part of the uterine wall at an advanced stage of allantoic attachment. The arrow points to a hollow trophoblastic villus (× 260). **16.** Part of the superficial region of the uterine wall of late allantoic attachment. Maternal blood capillaries (arrow) has a distinct lining of enlarged endothelial cells (arrowhead) (× 260). **17.** Part of the deep region of the uterine wall at late allantoic attachment to show pools of extravasated maternal blood (arrows) (× 350). **18.** Section of the uterus with an embryo in early limb-bud stage of development. A part of the yolk sac is still applied to the uterine wall (arrow). Diffuse allantoic placenta (arrowheads) occur in the rest of the uterus (× 18).

Figures 19–25. **19.** Part of the free folded yolk-sac splanchnopleure at early limb-bud stage. Note the endodermal layer (arrow) with hypertrophied cells (× 100). **20.** Part of the diffuse placenta at early limb-bud stage to illustrate the occurrence of numerous hollow invasions of the basal trophoblast (arrows) into the syncytiotrophoblastic zone (× 80). **21.** Part of the placenta to show maternal blood capillaries (arrows) with distinct endothelial lining with hypertrophied endothelial cells (× 260). **22.** Part of the maternal symplasma in the deeper region of the uterine wall at late limb-bud stage. Arrow points to pools of extravasated maternal blood (× 260). **23.** Section of the uterus with an embryo in late limb-bud stage. The yolk-sac splanchnopleure (arrow) is free on all the sides (× 10). **24.** Part of the diffuse placenta at late limb-bud stage. Note placental tubules (arrows) containing maternal blood capillaries which have darkly stained walls. In some places the placenta has formed a network. Multinucleate endometrial cells (arrowhead) occur on the maternal border of the placental labyrinth (× 100). **25.** Part of the diffuse placenta at late limb-bud stage. Note the distinct endothelium (arrow) in the maternal blood capillaries (× 180).

Figures 26–31. **26.** One of the lobes (shaded arrow) composed of a labyrinth of placental tubules in the discoid placenta at late limb-bud stage. Allantoic mesenchyme occurs between adjacent lobes and between the placental tubules (arrowhead) (× 80). **27.** Section of the uterus at mid-pregnancy. Please see text for description. Thin arrow points to the mesometrially located discoid placenta with a deep cleft in the center. Arrowhead points to the diffuse placenta. A thick arrow points to the cavity of the free yolk sac (× 8). **28.** Part of the yolk-sac splanchnopleure at mid-pregnancy. Arrow points to endodermal cells and arrowhead points to mesodermal cells (× 200). **29.** Part of the diffuse placenta at mid-pregnancy. Arrow points to maternal blood capillaries in the placenta (× 240). **30.** Section of the uterus at full term. Arrow points to the definitive placental disc. Arrowhead points to the collapsed yolk sac (× 6). **31.** Part of the collapsed yolk sac at full term. Note the presence of extravasated blood (arrow) on one side of the yolk sac (× 52).

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Gel electrophoretic studies with reference to functional morphology of the salivary glands of *Acanthaspis pedestris* Stal. (Insecta: Heteroptera: Reduviidae)

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Abstract. Histological profile of the anterior and posterior lobes of the principal salivary and accessory glands of the reduviid *Acanthaspis pedestris* Stal. has been discussed in relation to enzyme pattern of the posterior lobe and the zootoxic substances of the anterior lobe as well as the protein fractions of the salivary gland lobes and the haemolymph.

Keywords. Reduviidae; principal gland; accessory gland; hilus; zootoxic; watery saliva; protein fraction; R_m value.

1. Introduction

The structure and functional diversity of the salivary glands in the different groups of Hemiptera were analysed by Baptist (1941) and the importance of cecidogenetic and disease transmitting ability of the phytophagous Heteroptera in relation to the structure and function of the salivary system have been highlighted by Bronskill *et al* (1958), Miles (1959, 1964a, b, 1967, 1968, 1972), Salkeld (1960), Miyamoto (1961), Saxena (1963) and Hori (1969). Wigglesworth's (1943) description of the morphology of the salivary gland of Rhodnius suggesting the presence of haemalbumen like pigments, was followed by an analysis by Edwards (1961) of the functions and biochemical components of the salivary gland in *Platymeris rhadamanthus* Gaerst. The variation in salivary gland morphology of 9 sub families of Reduviidae established the inter-species diversity among Reduvoidea (Louis and Kumar 1973). Haridass and Ananthakrishnan (1981) illustrated the structure and functional morphology of the salivary glands of the subfamilies Piratinae, Echtrichodinae and Triatominae (Reduviidae). The present paper highlights the functional morphology of the different lobes of salivary gland along with histological details and electrophoretic analysis of diverse proteins involved in the salivary system of the predatory bug *Acanthaspis pedestris* Stal.

2. Materials and methods

Adults and nymphs of *A. pedestris* were collected from the semiarid zones of Kanyakumari district (Tamil Nadu) and a culture was maintained successfully under laboratory conditions. The adult insects were separated from the mass culture and utilized for the following experiments. The starved insects were etherized and the salivary system was carefully dissected out using insect ringer, fixed in alcoholic Bouin's solution and subsequently dehydrated through the alcohol series for embedding. Sections were made (7 μ) and stained with Delafields haematoxylin and eosin.

Toxic effects of secretion from different lobes of the salivary system were tested. Starved insects were etherized and dissected to expose the salivary glands. Using a graduated needle like glass capillary tube, 25 μl of the secretions were drawn directly and separately from the anterior, posterior and accessory lobes. These secretions were gradually injected into the thorax in the region of the pleural membrane of the hind coxal base of the test insect, *Oxya nitiduala* (Walker) (Acrididae). Using a stop watch, the time required for the arrest of the movements of the legs, tarsal segments, wings, respiratory movements of the abdomen and the total paralysis of the test insects was calculated. Twenty-five μl of diluted (GD water 1:2) salivary extracts obtained separately from known weight of the different salivary lobes dissected out from etherized insects were also tested in a similar way. Salivary secretions extracted in a similar manner, diluted with distilled water, but boiled at 50°C for 10 min in a water bath were used as control. The qualitative protein profiles of salivary glands and haemolymph, through polyacrylamide gel electrophoresis were identified (Davis 1964) on 7·5% tube gels of 11 cm by adjusting the current to 2·5 mA, per tube for about 3 h at 4–10°C, until the tracer dye migrated to a distance of about 10 cm. The samples (50 μl) were injected carefully into the gel tube using Finn pippete digital (Finland). The gels were removed and stained with 0·02% Coomassie brilliant blue in a mixture of methanol, acetic acid and water (25:7:68) for 24 h and destained for 48 h. The gels were stored in 7% acetic acid and were scanned by LKB 2202 ultroscan laser densitometer (Sweden) and the electropherograms were recorded by using the recording integrater LKB 2220 Bromma (Sweden). The different protein fractions were identified by computing the R_m values of the proteins using the following formula:

$$R_m \text{ value} = \frac{\text{Distance travelled by the sample}}{\text{Distance travelled by the dye}}.$$

3. Results

3.1 *Histological profile*

The small caplike anterior lobe and a long tubular posterior lobe of the principal salivary gland of *A. pedestris* is separated by a distinct hilus (figures 1A and C). The accessory glands attached to the lateral sides of the first midgut appear triangular with a tubular appendix opening into the common salivary duct (figure 1G). Both the lobes of the principal salivary gland comprise of single layer of oval shaped cells. The uninucleate anterior lobe cells are devoid of vacuoles having a few granular materials with less viscous cytoplasm, whereas the posterior lobe cells have two nuclei with highly viscous granular cytoplasm (figure 1B, D, E). The holocrine salivary secretion of both anterior and posterior lobes (figure 1B, F), is separately stored in the spacious lumen. The anterior part of both lobes reveal increased secretory activities. The salivary lobes open out individually by a small pore, guarded by thick circular muscles, into a compartmentalised hilus (figure 1C). The accessory gland cells are uninucleate with less viscous cytoplasm and secretes a watery fluid which is stored in its flattened lumen (figure 1H). A well developed,

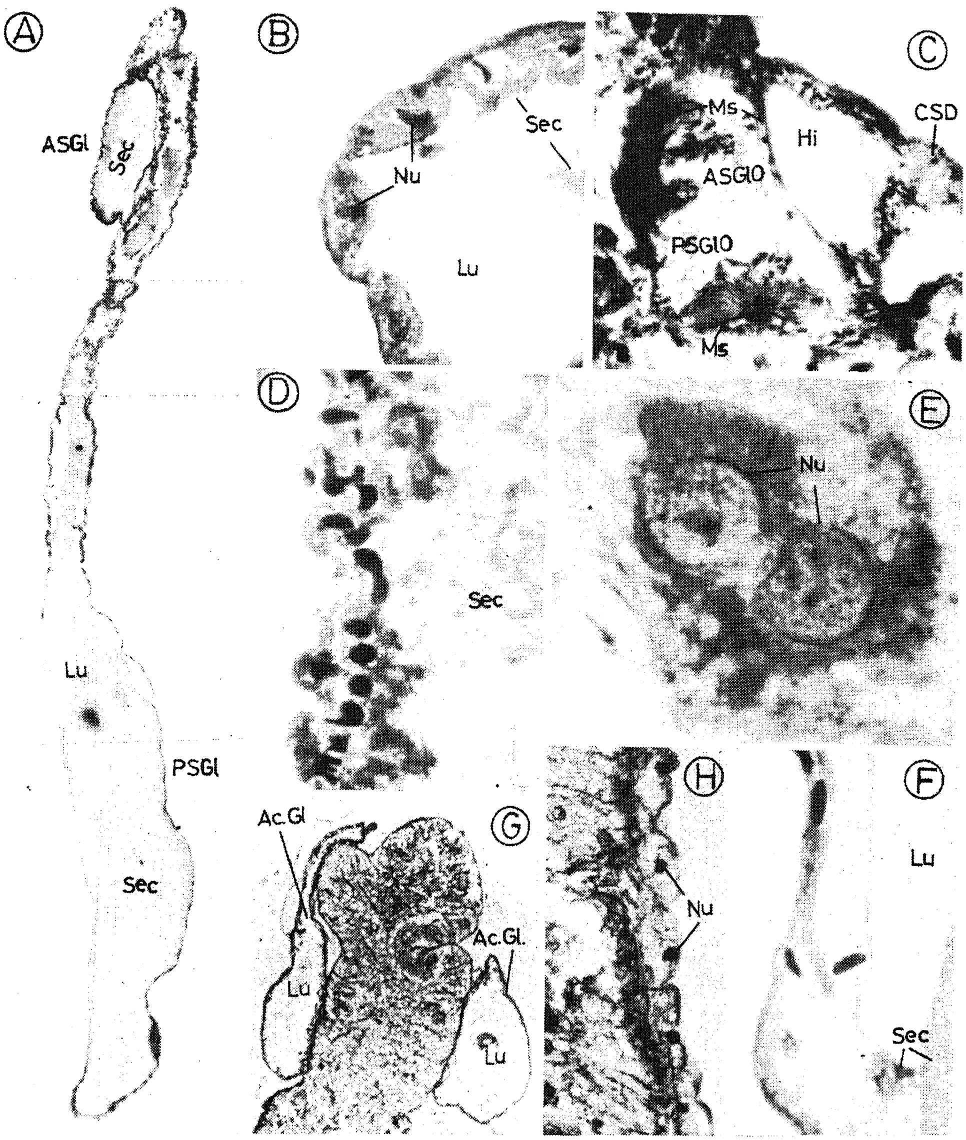

Figure 1. Histology of the salivary system in *A. pedestris*. **A.** LS of the principal salivary gland (×22). **B.** Uninucleate cells of anterior salivary lobe (×222). **C.** LS of hilus of salivary lobes (×200). **D.** Posterior salivary lobe (portion enlarged) (×262). **E.** Binucleate cells of posterior lobe (×782). **F.** Posterior salivary gland showing holocrine secretion (×225). **G.** Accessory salivary gland (×24). **H.** Uninucleate cells of accessory gland (×175).
(ASGl, Anterior salivary gland; PSGl, posterior salivary gland; Ac.Gl, accessory gland; Sec, secretory materials; Lu, lumen; Nu, nucleus; Hi, hilus; Ms, muscle layers; ASGlo, anterior salivary gland opening; PSGlo, posterior salivary gland opening; CSD, common salivary duct).

compartmentalised hilus with muscular valve at the junction of the anterior and posterior lobes of the principal salivary gland communicates with the accessory glands, opening into the common salivary duct.

3.2 *Zootoxic effect*

Table 1 indicates the zootoxic effect of concentrated as well as diluted saliva of anterior lobe, posterior lobe, accessory glands and the control. The anterior lobe secretion immobilized the test insect faster than the posterior lobe secretion whereas the accessory gland secretion did not show any effect.

3.3 *Gel electrophoresis studies*

The zymograms and densitometric electropherograms (figure 2) confirm the total number of protein fractions such as 6, 11, 2 and 9 in the anterior and posterior salivary lobes, accessory gland and haemolymph respectively. The banding pattern

Table 1. Toxicity in the secretion of different lobes of salivary gland of *A. pedestris.*

Organ	Time required to immobilize the acridid *Oxya nitidula* (Walker)	
	Saliva	Dilute saliva
Anterior lobe	69 ± 3 s	532 ± 49 s
Posterior lobe	289 ± 50 s	1591 ± 54 s
Accessory gland	No effect	No effect
Control	No effect	No effect

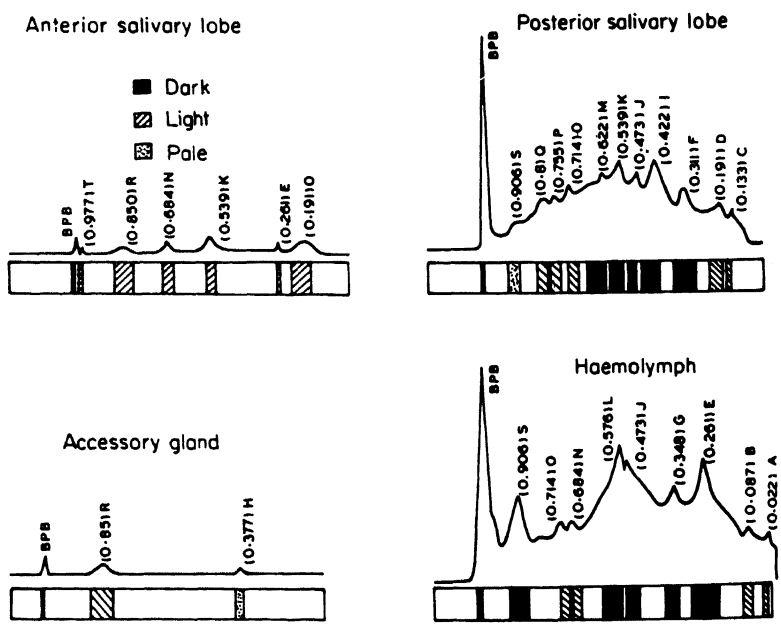

Figure 2. Densitometric scan of protein fractions in salivary system and Haemolymph of *A. pedestris.*

of the histogram as well as the peak obtained in the chromatograms indicate the protein concentration, with most of the bands relating to the haemolymph and posterior salivary lobe being dark, while those of the anterior lobe are light, and those of the accessory gland being pale. Table 2 illustrates the percentage composition of the protein fractions in the salivary system and the haemolymph. The protein fractions 'E' and 'N' in the anterior lobe as well as 'J', 'O' and 'S' in the posterior lobe are found to be similar to the haemolymph proteins, while the other protein fractions differ from each other. In the anterior and posterior salivary lobes the proteins 'K' and 'D' appear to be identical.

4. Discussion

The salivary systems of Reduviids with diverse morphology are found in the different sub-families. *A. pedestris* have a bilobed principal gland and elongated accessory gland as those of the members of the sub-families Harpactorinae, Piratinae, Rhaphidosomatinae and Salyavatinae (Haridass and Ananthakrishnan 1981). Despite their similarity in the morphology there are histological and functional variations in the salivary gland of *A. pedestris*. While Haridass and Ananthakrishnan (1981) earlier indicated binucleate nature of the cells with apocrine and merocrine in anterior and posterior lobes respectively, present observations indicate uninucleate cells with merocrine nature of the secretion in the anterior lobe and the binucleated cells with holocrine nature of secretion in the

Table 2. Electrophoretic analysis of protein profiles of Haemolymph and salivary glands of *A. pedestris*.

Protein (R_m value)	Haemolymph	Salivary apparatus: Anterior lobe	Posterior lobe	Accessory gland
(0·022) A	0·752	—	—	—
(0·087) B	3·312	—	—	—
(0·133) C	—	—	0·427	—
(0·191) D	—	46·453	3·655	—
(0·261) E	18·572	3·449	—	—
(0·311) F	—	—	6·936	—
(0·348) G	14·515	—	—	—
(0·377) H	—	—	—	9·816
(0·422) I	—	—	15·872	—
(0·473) J	17·439	—	10·268	—
(0·539) K	—	5·434	20·72	—
(0·576) L	31·301	—	—	—
(0·622) M	—	—	23·194	—
(0·684) N	3·42	16·114	—	—
(0·714) O	2·024	—	6·733	—
(0·755) P	—	—	4·345	—
(0·8) Q	—	—	6·963	—
(0·85) R	—	25·339	—	90·183
([illegible]) S	[illegible]	[illegible]	[illegible]	[illegible]

posterior lobe of the principal salivary gland. Observations from the zootoxicity experiments and the diverse functions of the secretions were further confirmed by the presence of the different protein fractions in the anterior, posterior and accessory glands of the salivary system. This result deviates from the earlier analysis made by Edwards (1961) in the different lobes of salivary gland of the reduviid, *Platymeris rhodhamanthus* Gaerst., wherein he confirmed the presence of zootoxic substances both in the anterior as well as posterior lobes besides the digestive enzyme secreted by both the lobes. The accessory glands differ histologically from the lobes of the principal gland and secretes watery saliva, which has less protein fraction than the other lobes. Similar results were highlighted recently in pentatomid and coreid bugs (Miles and Slowiak 1976) and in assassin bugs (Haridass and Ananthakrishnan 1981). The presence of identical proteins in the haemolymph as well as anterior and posterior lobes presumably indicate the transportation of haemolymph protein into the salivary system. This observation coincides with the results obtained in phytophagous heteropteran by Miles (1967), who stated that haemolymph transports the essential amino acids, glycerol and glucose to the saliva secretion.

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Sex-related biochemical investigation of the diaptomid, *Heliodiaptomus viduus* Gurney (Crustacea: Copepoda)

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Abstract. The freshwater diaptomid, *Heliodiaptomus viduus* was subjected to biochemical analysis to study its sex-related differences. Its chemical composition was similar to that of other copepods, the concentration of the components decreased in the order of protein > lipid > carbohydrate. The ovigerous females show higher dry weight and consequently the protein, lipid and carbohydrate concentrations are greater than non-ovigerous females and males. There are also considerable qualitative and quantitative differences in the free amino acid composition.

Keywords. *Heliodiaptomus viduus*; sex-related biochemical composition; freshwater tropical copepod.

1. Introduction

Although estimation of biochemical composition of freshwater micro- and macro-crustaceans was attempted in the past (e.g. Michael and Chandran 1967; Rajendran 1973), following the method of Birge and Juday (1922) on freshwater plankton to understand their metabolism, no efforts were made to distinguish males from females and ovigerous females from non-ovigerous females. Altaff and Chandran (1988) carried out an electrophoretic analysis of the body homogenate of *Heliodiaptomus viduus* to distinguish the protein fractions of males, females and ovigerous females. The present study is an attempt to find out the sex-related differences in protein, carbohydrate, lipid and free amino acid composition of the body homogenate of *H. viduus*. The copepod was chosen for detailed investigation as it forms an important constituent of the biota of most of the freshwater bodies of south India (Rajendran 1973).

2. Materials and methods

Zooplankton were collected from the Chetput pond of the Hydrobiological Station, Tamil Nadu State Fisheries Department, Madras, using bolten silk plankton net and maintained in filtered pond water in the laboratory. In the present study biochemical composition of males, females and ovigerous females of *H. viduus* has been determined using 5 sets of samples. For each analysis about 100 mg of 24 h starved males, females and ovigerous females were separated under a binocular dissection microscope and pooled. They were then transferred on to a tarred bolten silk piece and the excess moisture removed using a filter paper as suggested by Rajendran (1973). The moisture-free animals were weighed in a monopan balance. This procedure was uniformly followed for all the samples.

To estimate water and dry tissue content, the samples were dried in a hot air oven at 60°C to a constant weight. The colorimetric procedure of Lowry *et al* (1951) was suitably adopted for protein estimation and the colour intensity of the solution was measured at 520 nm. Different concentrations of bovine serum albumin served as the standard.

Qualitative analysis of free amino acids was carried out following the method of Smith (1968). Descending double dimension chromatogram was run using solvent I (butanol, glacial acetic acid and distilled water in the ratio of 12:3:5) for the first run and solvent II (160 g phenol, 40 ml distilled water and 1 ml of 25% ammoniacal solution) for the second run. Ninhydrin (0·2%) was used to detect the amino acids. A standard chart of amino acid was prepared using commercially available amino acids. For quantitative estimation of free amino acids the volume of aqueous layer of free amino acid was measured and 100 μl of this was spotted on the chromatographic paper. To prepare the standard, 1 mg of each commercially available amino acid was weighed and dissolved in 12 ml of 80% ethanol and 20 μl of this was spotted and double dimension chromatogram was run. Immediately after staining, the amino acid bands were eluted in 3 ml of 50% acetone and the optical density measured at 420 nm for proline and at 530 nm for all other amino acids.

Carbohydrates were estimated following the method of Roe (1955). Anthrone reagent was used and the colour developed was measured at 620 nm. Glucose was used as the standard.

Lipid was extracted according to the method of Folch *et al* (1957) and the estimation was followed as described by Barnes and Blackstock (1973). Sulphophosphovanillin was used as reagent and the colour developed was read at 520 nm (Spectronic 21 model of Bausch and Loumb). Statistical analysis of the data was performed using the Student's t test.

3. Results and discussion

The mean values of biochemical components of males, females and ovigerous females of *H. viduus* were found to be 65·08% of protein, 14·42% of lipid and 4·55% of carbohydrate. These values conform to those of other copepods in general (Birge and Juday 1922; Raymont *et al* 1964; Rajendran 1973). However, the males, females and ovigerous females of *H. viduus* show considerable variation in their biochemical composition. The mean protein, lipid and carbohydrate levels in males, females and ovigerous females are presented in figure 1.

Statistical analysis for the test of significance with reference to water content, protein, lipid and carbohydrate in males and non-ovigerous females indicates that the difference is not significant. However there is a significant difference ($P < 0{\cdot}001$) in protein and lipid content of males as well as non-ovigerous females when compared to the ovigerous females. The ovigerous females exhibit significant differences with respect to water and carbohydrate content also ($P < 0{\cdot}01$) when compared with that of males and non-ovigerous females (table 1). The lower water content (1%) and higher values of protein, lipid and carbohydrate of ovigerous females may be attributed to the reproductive phase of the ovigerous females in general and to the eggs in particular. It is known that the yolk material of the egg is rich in protein, lipid and carbohydrate in many crustaceans (Barnes 1965), which qualifies it to serve as a reserve food material for embryo development. The higher

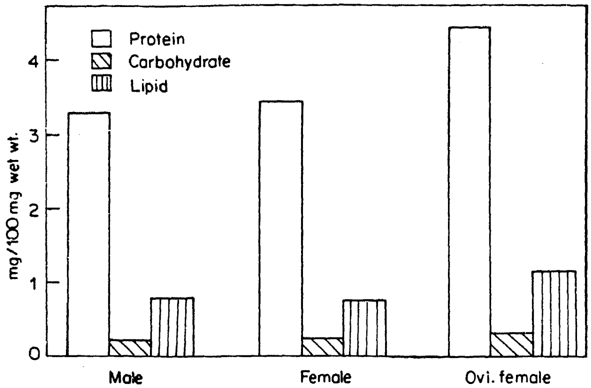

Figure 1. Variation in the biochemical components of male, female and ovigerous female of *H. viduus*.

Table 1. Biochemical components of males, females and ovigerous females of *H. viduus* ($X \pm SD$).

	Water content	Protein (mg/100 mg wet wt)	Carbohydrate (mg/100 mg wet wt)	Lipid (mg/100 mg wet wt)
Male	94·81 ± 0·33	3·27 ± 0·21	0·21 ± 0·04	0·78 ± 0·04
Female	94·73 ± 0·36	3·43 ± 0·16	0·24 ± 0·03	0·76 ± 0·03
Ovigerous female	93·78 ± 0·31*	4·45 ± 0·45**	0·32 ± 0·04*	1·16 ± 0·13**

Values with asterisk are statistically significant.
*$P < 0{\cdot}01$; **$P < 0{\cdot}001$.

values of the biochemical components of ovigerous females in the present study conform to the earlier report of glycolipoprotein yolk in *H. viduus* (Altaff and Chandran 1988).

As in other crustaceans, *H. viduus* also shows higher concentrations of free amino acids. However, the concentration of free amino acids in this animal is less when compared to that of *Calanus finmarchicus* (Schoffeniels and Gilles 1970) which may be due to the habitat difference. A noticeable difference is found in the quantity of free amino acids of males, females and ovigerous females (table 2). The difference in the free amino acid content of ovigerous and non-ovigerous females may be due to the higher free amino acid content of the yolk found in the eggs of ovigerous females. Chromatographic separation further reveals the presence of 8 free amino acids in the male and female whereas 12 in the ovigerous female. Out of the 8 amino acids proline, aspartic acid, glycine, iso-leucine and leucine are the 5 amino acids common to both males and females. The other 3 amino acids are lysine, histidine and valine in males whereas serine, threonine and alanine in females. All the 8 amino acids occurring in females are also noticed in the ovigerous females, in addition to these amino acids histidine, lysine, arginine and phenylalanine also occur in the ovigerous females.

Table 2. Free amino acids and their concentration in the male, female and ovigerous female of *H. viduus*.

	μg/100 mg wet weight		
Amino acid	Male	Female	Ovigerous female
Histidine	2·04	—	1·22
Lysine	1·21	—	2·23
Arginine	—	—	2·04
Proline	6·59	6·54	9·63
Aspartic acid	2·72	2·39	12·72
Serine	—	2·72	5·15
Glycine	3·33	9·03	3·63
Threonine	—	1·81	3·03
Alanine	—	1·21	2·72
Phenylalanine	—	—	5·45
Valine	1·12	—	—
Isoleucine	1·18	2·72	5·45
Leucine	3·15	1·36	1·59

From the available data on crustacean metabolism, it may be suggested that the oxidative metabolism in crustacea can differ considerably from the commonly accepted pattern in other organisms. Such data prompted Waterman (1960) to come to the tentative conclusion that though carbohydrate may be the main metabolic substrate in some species, more often a mixture of protein, carbohydrate and lipid is needed. The large amount of free amino acids in crustacean tissues and the variability of its quantity under conditions leading to an energetic adjustment may perhaps be considered as indicating the use of amino acids in the process of energy production.

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Energy transfer efficiency in three hymenopteran egg parasitoids of *Homoeocerus prominulus* (Tagus) (Insecta : Heteroptera : Coreidae)

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Abstract. Energy budgets of the solitary egg parasitoids, *Anastatus ramakrishnae* (Mani) and *Trissolcus* sp., and a gregarious egg parasitoid, *Xenoencyrtus* sp. near *niger* Riek on the eggs of *Homoeocerus prominulus* have been compared. Energy allocation for consumption, assimilation and metabolism were greater in the gregarious parasitoid than in the solitary parasitoids; on the other hand, higher production efficiency and lower assimilation efficiency were noticed in the solitary parasitoids than in the gregarious parasitoids. The rate of consumption, assimilation, production and metabolism of gregarious and solitary parasitoids have been compared in the light of their reproductive efficiencies.

Keywords. Bioenergetics; hymenopteran parasitoids; coreid host.

1. Introduction

Food utilization efficiency among parasitoids is high because they consume limited food resource of a seemingly high nutritional quality and their presumed relative inactivity within the host (Slansky and Scriber 1985). In general, gregarious parasitoids inevitably entail internal competition for food resource provided by the host, and this is a physiologically intricate interaction. In the host-parasitoid systems where a single parasitoid larva consuming the nutrients present in the whole body of the host is relatively simpler in terms of energy allocation, ingestion can be estimated from the energy content of the host (Howell and Fisher 1977). Arthur and Wylie (1959), Varley (1961) and Weseloh (1969) have discussed the food conversion efficiency of hymenopteran parasitoids which are generally known to be very efficient in utilizing and assimilating the biomass of their hosts into their tissues. Only in a few cases energy budget have been made for solitary parasitoids (Chlodny 1968; Edgar 1971; Cameron and Redfern 1974; Howell and Fisher 1977) and hyperparasitic parasitoids (Prakash and Pandian 1978). The present study aims at determining energy budget of 3 hymenopteran chalcidoidean parasitoids, *Anastatus ramakrishnae* (Mani) (Eupelmidae) and *Trissolcus* sp. (Scelionidae), solitary parasitoids and *Xenoencyrtus* sp. near *niger* Riek (Encyrtidae), a gregarious parasitoid, of the eggs of *Homoeocerus prominulus*.

2. Materials and methods

A. ramakrishnae, *Xenoencyrtus* sp. and *Trissolcus* sp. were reared in the laboratory by providing cotton wads soaked in dilute honey. To maintain a constant supply of host eggs for the parasitoid, *H. prominulus* was reared *en masse* in the laboratory by providing fresh host plants (*Prosopis spicigera* L.) daily.

The eggs of the coreid bugs were exposed in closed plastic vials (10 × 8 cm) to enable parasitisation. Newly emerged adult parasitoids were collected and their live

weights were taken, and dried at $60 \pm 5°C$. The parasitoids that emerged from host egg shells were dissected and the meconia were separated. The particles of the meconia and the egg shells were weighed and dried for the calorific determination as follows:

Following the gravimetric method of Waldbauer (1968), food consumption (C), egestion (FU) and growth (P) were estimated. Energy contents of the normal egg, meconia, empty parasitised egg shells of the host as well as that of the adult parasitoids were estimated in a Parr 1421 semi-micro bomb calorimeter, following the standard procedure. Using these energy values, the mass budget involving C, FU and P was evaluated into energy budget in J/mg/individual.

Bioenergetic parameters were estimated following the IBP formula of Petrusewicz and MacFadyen (1970):

$$C = FU + P + R.$$

Assimilation was calculated as the difference between food consumed and that of faeces:

$$A = C - FU.$$

Whereas metabolism was calculated as the difference between assimilated energy and that of growth:

$$M = A - P.$$

Rates of consumption (Cr), assimilation (Ar), production (Pr) and metabolism (Mr) were calculated by dividing the respective quantitative values expressed on per insect basis by the mid-body weight of the insect (mg)—in this study it was based on the weight of the host egg (mg), and duration of the immature stage expressed in J/mg live insect/day. The following formulae were used to compute the rates and efficiencies:

$$Cr = \frac{\text{Consumption (J/insect)}}{\text{Mid-body weight (mg)} \times \text{duration of the insect/stage}}.$$

$$Ar = \frac{\text{Assimilation (J/insect)}}{\text{Mid-body weight (mg)} \times \text{duration of the insect/stage}}.$$

$$Pr = \frac{\text{Production (J/insect)}}{\text{Mid-body weight (mg)} \times \text{duration of the insect/stage}}.$$

$$Mr = \frac{\text{Metabolism (J/insect)}}{\text{Mid-body weight (mg)} \times \text{duration of the insect/stage}}.$$

$$Ase\ (\%) = \frac{\text{Assimilation (J/insect)}}{\text{Consumption (J/insect)}} \times 100.$$

$$Pe_1\ (\%) = \frac{\text{Production (J/insect)}}{\text{Assimilation (J/insect)}} \times 100.$$

$$Pe_2\ (\%) = \frac{\text{Production (J/insect)}}{\text{Consumption (J/insect)}} \times 100.$$

3. Results

Energy budgets for the adult, meconium and empty host egg shell of *A. ramakrishnae*, *Trissolcus* sp. and *Xenoencyrtus* sp. and zero day egg of host, *H. prominulus* are summarised in table 1. Efficiency to utilize the available energy content of host egg (0·541 J/mg/individual) varied with the parasitoids. The energy values obtained for the meconium were strikingly different; the meconia of *A. ramakrishnae* (dark brown coloured, single and folded mass) had an energy value of 0·203 J/mg/individual while it was 0·216 J/mg/individual in *Trissolcus* sp. (pale yellowish, single mass without fold), and 0·169 J/mg/individual in *Xenoencyrtus* sp. (pale brown with orange tinged, small granules in appearance).

Comparing consumption, assimilation, metabolism and efficiencies of assimilation and production among the 3 species (tables 2 and 3), maximum food consumption of 0·484 J/mg/individual/day was evident in *Xenoencyrtus* sp. as compared to *A. ramakrishnae*, while it was least in *Trissolcus* sp. (0·447 J/mg/individual/day). Assimilation of energy by parasitoids also followed a trend similar to that of consumption. More energy assimilation was recorded for *Xenoencyrtus* sp. (0·315 J/mg/individual/day) while it was comparatively low in *A. ramakrishnae* and *Trissolcus* sp. (0·278 J/mg/individual/day and 0·261 J/mg/individual/day respectively).

Table 1. Energy budgets for the eggs of *H. prominulus* and their parasitoids.

Material	Energy value (J/mg/individual)
Host	
Freshly laid egg	0·541 ± 0·18
Parasitoids	
Anastatus ramakrishnae	
Adult	0·262 ± 0·086
Meconia	0·203 ± 0·09
Empty host egg shell	0·060 ± 0·012
Xenoencyrtus sp.	
Adult	0·183 ± 0·126
Meconia	0·169 ± 0·11
Empty host egg shell	0·057 ± 0·019
Trissolcus sp.	
Adult	0·247 ± 0·072
Meconia	0·216 ± 0·096
Empty host egg shell	0·064 ± 0·015

Table 2. Energy budgets of 3 parasitoids in the host eggs of *H. prominulus*.

	Energy value (J/mg/individuals/day)		
Parameters	*A. ramakrishnae*	*Xenoencyrtus* sp.	*Trissolcus* sp
Consumption	0·481 ± 0·012	0·484 ± 0·019	0·477 ± 0·015
Assimilation	0·278 ± 0·090	0·315 ± 0·110	0·261 ± 0·096
Metabolism or respiration	0·016 ± 0·007	0·132 ± 0·017	0·014 ± 0·006

Table 3. Assimilation and production efficiencies of 3 parasitoids of the host eggs of *H. prominulus*.

Parameters	Energy value (%)		
	A. ramakrishnae	*Xenoencyrtus* sp.	*Trissolcus* sp.
Assimilation efficiency (Ase)	57·80 ± 8·16	65·08 ± 18·21	54·72 ± 10·18
Production efficiency			
Pe_1	54·47 ± 12·04	37·81 ± 20·13	51·78 ± 13·24
Pe_2	94·25 ± 4·62	58·10 ± 24·07	94·64 ± 4·13

Table 4. Rate of energy transfer in 3 egg parasitoids of *H. prominulus*.

Energy parameters	Energy value (J/mg/day)		
	A. ramakrishnae	*Xenoencyrtus* sp.	*Trissolcus* sp.
Cr	5101·52 ± 12·0	3483·33 ± 19·3	3975·0 ± 15·4
Ar	2948·49 ± 90·2	2267·05 ± 11·3	2175·0 ± 96·4
Pr	2778·79 ± 86·0	1940·91 ± 126·0	2058·3 ± 72·0
Mr	169·70 ± 7·3	950·0 ± 17·1	116·67 ± 6·4

Assimilation and production efficiencies (table 3) indicated that efficiency of assimilation was more in the case of gregarious parasitoid than in solitary parasitoids while production efficiency was more in solitary parasitoids than in gregarious parasitoid.

High consumption and assimilation rates were recorded in *A. ramakrishnae* (5101·52 and 2948·49 J/mg/day respectively) and *Trissolcus* sp. (3975 and 2175 J/mg/day respectively) whereas in *Xenoencyrtus* sp. it was only 3483·33 J/mg/day and 2267·05 J/mg/day for the consumption and assimilation rates respectively. While considering the metabolic rate, *Xenoencyrtus* sp. spent more energy (950 J/mg/day) than the other two parasitoids, *A. ramakrishnae* (169·7 J/mg/day) and *Trissolcus* sp. (116·67 J/mg/day) (table 4).

4. Discussion

Energy budgets hold the key for understanding reproductive strategies of individuals (Price 1974; Boggs 1981). Since hymenopteran parasitoids tend to exhibit high food utilization efficiency, it is essential to study the amount of energy being utilized by the parasitoid for their complete development. The results presented in this study reveal the variation between the energy expenditure of two solitary egg parasitoids, *A. ramakrishnae* and *Trissolcus* sp. besides a gregarious parasitoid *Xenoencyrtus* sp. These results support the view of Smith and Smilowitz (1976) that the nutritional demands imposed by the individuals of gregarious parasitoids are greater than those of an individual solitary parasitoid. Since the amount of food ingested by the parasitoid larvae is important for survival, one would expect the mortality rate to increase with both the number of eggs laid per host and the hatching time between larvae. Although parasitising the same host egg, adults of *A. ramakrishnae* and *Trissolcus* sp. completed their development within 14 and 11

days respectively, whereas adults of *Xenoencyrtus* sp. completed its development within 9·5 days itself. This faster post-embryonic developmental period of *Xenoencyrtus* sp. might be advantageous for them to avoid mortality due to the depletion of nutrients during development. The importance of hatching time and survival of the gregarious parasitoid, *Trichogramma embryophagum* and *T. pretiosum* was explained by Klomp *et al* (1980); Strand and Vinson (1985). More energy was spent by the gregarious parasitoid, *Xenoencyrtus* sp. for consumption, assimilation and respiration whereas it was low in the solitary parasitoids. But in this regard Chlodny (1968) found that approximately 70% of *Pieris brassicae* pupa was consumed by a solitary larva of the pupal parasitoid, *Pimpla instigator* or by about 44 larva of the gregarious *Pteromalus puparium*, suggesting relatively high exploitation efficiencies for both solitary and gregarious parasitoids. Quantities of the egesta and assimilated food depend on the efficiency of the digestive and absorptive machinery (Muthukrishnan and Pandian 1986). Therefore, high energy expended in *A. ramakrishnae* and *Trissolcus* sp. for egestion as meconia caused the decrease in the energy value for efficiency of assimilation, whereas in *Xenoencyrtus* sp. low energy expenditure for egestion is presumably due to partitioning of food caused by lesser food consumption resulting in an increase in the energy for assimilation. The energy budget obtained for *Xenoencyrtus* sp. when compared with two species indicates a low production efficiency because of the undersized adults. Egg parasitoids lack the opportunity of increased food intake from the host and if the weight of the host is less than that required by the parasitoid to achieve its ideal body weight, then the resulting adult parasitoids may have a reduced body weight (Slansky 1986). Moreover ovipositing females of some parasitoids and their hyperparasitoids may assess host size and bias the sex ratio of the eggs they lay toward the smaller sized sex (usually males) in smaller hosts (Kfir and Rosen 1981a, b, c) as well as alter the number of eggs they lay per host (Luck *et al* 1982; Charnov and Skinner 1984). High assimilation efficiencies (55–94%, $\bar{x} = 68\%$), have been reported for more than 15 species of parasitoids (Slansky 1986). Results presented in this study, also indicate the higher metabolic rate in *Xenoencyrtus* sp. in comparison to *A. ramakrishnae* and *Trissolcus* sp. and one possible attribute is that the gregarious adults spend more energy for respiration than the solitary ones.

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Glycerol formation in silkworm eggs

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Abstract. The temporal pattern of accumulation of glycerol was examined during the early diapause period in silkworm eggs. Studies on the activities of two important enzymes viz. NADP-dependent glycerol dehydrogenase and NADP-dependent glycerol phosphate dehydrogenase showed that the latter may be more important than the former in the production of glycerol during diapause in silkworm eggs.

Keywords. Glycerol formation; silkworm eggs.

1. Introduction

Conversion of glycogen to glycerol and sorbitol is known to occur during diapause in a number of insects including the silkworms *Bombyx mori* (Chino 1957a, b) and *Cecropia* (Wyatt and Meyer 1959). The glycerol formation which could be of physiological significance is widely evident (Asahina 1969). For instance, the diapause pre-pupa of *Bracon cephi* which can withstand $-40°C$ contains as much as 5 molal concentration of glycerol (Salt 1961). In certain species of insects, glycerol accumulation is highest when temperatures are lowest (Frankos and Platt 1976). While sorbitol has been widely accepted as a cryoprotective agent, the relative role of glycerol in diapause eggs of silkworm has not been examined.

Yaginuma and Yamashita (1978) showed that glycerol and sorbitol behave differently during diapause in silkworm eggs. While several studies have been carried out to examine the formation of sorbitol including the enzymes involved, nothing much is known about the formation of glycerol in silkworm eggs. The present studies were intended to examine the formation of glycerol during diapause and the activities of certain enzymes that may be responsible for the formation of glycerol in diapause, non-diapause and acid-treated artificial non-diapause eggs of silkworm.

2. Materials and methods

Bivoltine (NB_4D_2) and multivoltine (pure Mysore) races of the silkworm *Bombyx mori* L. were maintained under standard conditions. Eggs laid on polythene sheets were kept at $25 \pm 2°C$ with 75% rh.

For breaking diapause, 20 h old eggs were treated with HCl (specific gravity 1·075) at 46·1°C for 3–4 min, washed thoroughly with water and kept at $25 \pm 2°C$.

2.1 *Estimation of polyols*

Eggs weighing 0·5 g were homogenized in 5 ml of 80% ethanol using glass homogenizer. After centrifugation at 1000 *g* for 10 min the precipitate was washed

with 5 ml of 80% ethanol and dissolved in 1 ml of distilled water. From this solution, sorbitol and glycerol were separated by thin-layer chromatography following the method of Burton (1957) using butanol-acetic acid-water (4:1:2) as solvent.

2.2 *Enzyme preparation*

A 10% (w/v) homogenate of the eggs was prepared using a glass homogeniser fitted with teflon pestle. The homogenate was filtered through a cotton pad, centrifuged at 5500 *g* for 15 min at 0°C. The supernatant was filtered through Whatman No. 1 filter paper and the resultant filtrate used as the enzyme source.

2.2a *Assay of NADP-dependent glycerol dehydrogenase activity*: This was determined based on the method of Faulkner (1958). The reaction mixture consisted of 20 mM Tris-HCl buffer of pH 7·5, 4 mM $MgSO_4$, 10 mM dihydroxy acetone, 0·07 mM NADPH and 0·1 ml enzyme solution in a final volume of 1 ml.

The reaction was initiated by adding the substrate. The enzyme activity was determined by measuring the optical density at 340 nm. One unit of the enzyme activity was defined as the amount causing decrease of optical density by 0·01/min. Protein content was determined according to Lowry *et al* (1951) using bovine serum albumin standards.

2.2b *Assay of NAD-dependent glycerol phosphate dehydrogenase*: This was determined based on the method of Baranowski (1949). The reaction mixture consisted of 20 mM Tris-HCl buffer of pH 8·5, 4 mM $MgSO_4$, 10 mM dihydroxyacetone phosphate, 0·07 mM NADH and 0·1 ml of the enzyme extract in a final volume of 1 ml.

3. Results

3.1 *Polyol formation*

In non-diapause eggs, no glycerol could be traced. In diapause eggs, the level of glycerol was very low up to the 8th day after oviposition following which it

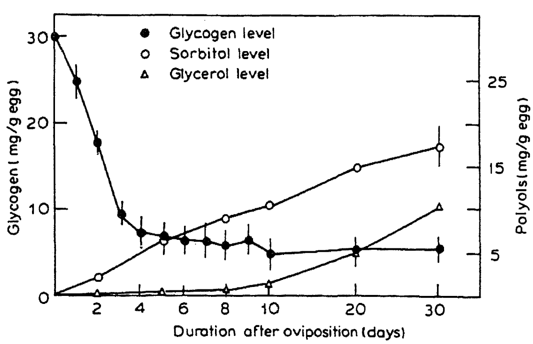

Figure 1. Changes in sorbitol and glycerol levels during diapause. Glycogen values are plotted for comparison.

increased significantly. In contrast, sorbitol level started increasing immediately after oviposition (figure 1).

3.2 *NADP-GDH activity*

This enzyme was found to be active in both diapause and non-diapause eggs. In non-diapause eggs, the activity was quite high at the time of oviposition. The activity further increased on the 2nd day following which it decreased reaching half the initial level during later stages of embryogenesis (figure 2). In diapause and acid-treated eggs, the activity was found to be similar to that observed in non-diapause eggs up to the 10th day. In diapause eggs, following the 10th day, the activity significantly increased and remained high for a long period.

3.3 *NAD-GPDH activity*

The activity of this enzyme could be detected in both diapause and non-diapause eggs. In non-diapause eggs, the activity decreased following oviposition and started

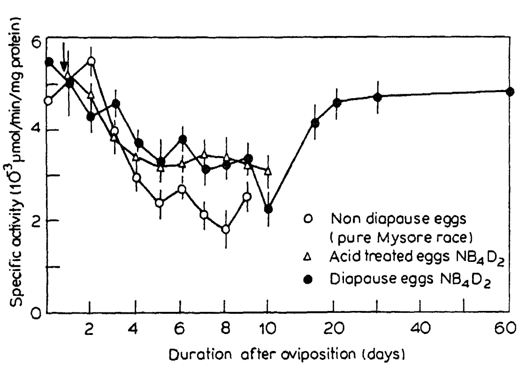

Figure 2. NADP-GDH activity in silkworm eggs. Arrow indicates acid treatment. Mean values with SD are plotted (n = 4).

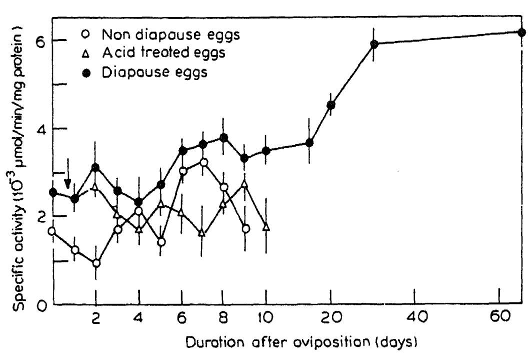

Figure 3. NAD-GPDH activity in silkworm eggs. Arrow indicates acid treatment. Mean values with SD are plotted (n = 4).

increasing again after the 2nd day reaching a peak on the 4th day. After a transient decrease on the 5th day, the activity started increasing again, reaching a higher peak on the 7th day following which, it decreased up to hatching (figure 3).

In diapause eggs, the activity was much higher than that in non-diapause eggs at the time of oviposition. The activity increased on the 2nd day and decreased up to the 4th day, increasing significantly thereafter. The increase was especially marked after the 10th day reaching twice the initial level by the 20th day. In acid-treated eggs, the pattern was comparable to that observed in non-diapause eggs, except that there is a temporal shift of about 2 days which is due to the delay in the initiation of development in these eggs.

4. Discussion

It is seen that glycerol accumulates with much delay following the accumulation of sorbitol. The significance of this delayed accumulation is not very clear. Earlier studies (Yaginuma and Yamashita 1978; Chandrashekar 1987) showed that glycerol level raises continuously and remains high even when the sorbitol level begins to drop in chilled diapause eggs after 40 days. It is likely that sorbitol acts as a major cryoprotective agent during the early phase of diapause while glycerol supplements sorbitol during later stages.

In both diapause and non-diapause eggs, the activity of NADP-GDH was high to start with and decreased as the age increased. After carefully examining the pattern of activity of this enzyme in the 3 types of eggs used, it can be said that while NADP-GDH may be contributing towards the formation of glycerol, it may not be a key enzyme controlling its production due to the following reasons. (i) The enzyme is equally active in both diapause and non-diapause eggs. (ii) There is no marked change in the level of its activity that could be correlated with the onset of diapause. (iii) The increase in its activity seen after the 10th day is in fact not very significant keeping in view, even higher levels observed at the time of oviposition. (iv) Acid treatment did not result in any marked change in the activity of this enzyme.

The enzyme NAD-GPDH was found to be quite active in both diapause and non-diapause eggs. The 2 peaks of activity observed during embryogenesis interestingly correspond to the blastokinesis and blue egg stages. It is quite possible that this enzyme plays an important role in embryonic development.

In diapause eggs, the activity of NAD-GPDH was clearly much higher than that observed in non-diapause eggs. The gradual increase in its activity up to the 10th day followed by a rapid increase closely parallels the pattern of increase of glycerol during diapause. Thus, the rapid increase in NAD-GPDH activity can account for the delayed accumulation of glycerol. Following acid treatment, the activity of this enzyme decreased considerably, from the initial high level to that comparable to what is observed in non-diapause eggs. Hence, it is suggested that NAD-GPDH may play a more important role than NADP-GDH in the formation of glycerol during diapause. The glycerolphosphate formed by the action of this enzyme may be converted to glycerol by removing phosphate either by a specific glycerophosphatase or by some phosphomonoesterase or any other general phosphate cleaving enzyme. If NAD-GPDH is accepted to be contributing mainly to the formation of glycerol, it can be visualised that the NADP requirement is minimised in the formation of glycerol.

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Sublethal effects of penfluron on the feeding physiology of *Papilio demoleus* Linn (Lepidoptera: Papilionidae)†

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Abstract. Topical and oral application of penfluron to *Papilio demoleus* Linn indicated that 7·6 μg of penfluron was needed through topical method to produce 50% mortality. Penfluron treatment caused a marked reduction in growth to a significant level of 42% in oral administration at dose 1·96 μg and 35% in topical application at sublethal dose 1·52 μg over that of control larva. The efficiency of *Papilio demoleus* to grow on citrus plant was very much affected.

Keywords. Penfluron; *Papilio demoleus*; growth rate; growth efficiency; food utilization.

1. Introduction

Papilio demoleus Linn (Lepidoptera: Papilionidae) is a serious pest of citrus plant, *Citrus limonum*. Studies on the damage potential by this pest to the citrus plant indicated a heavy rate of defoliation of plant to the tune of 4837 mg/g body weight of larvae in a day (Somasundaram 1985). The present study evaluates the potential use of penfluron, an analogue of chitin inhibitor diflubenzuron, through its effect on the food utilization efficiency and growth of *P. demoleus*.

2. Materials and methods

The larvae of *P. demoleus* were collected from the field and reared in the laboratory on leaves of *C. limonum* under LD 12:12 and temperature 30 ± 1°C at 80 ± 10% rh. 4 h old final instar larvae were used in the experiment.

2.1 *Bioassays*

Penfluron, chemically known as 1-(2, 6-diflurobenzoyl)-3-(4-trifluromethyl phenyl) urea was obtained as a 25% (w/w) wettable powder formulation from Duphar B V, Netherlands and used. Solutions of different strengths of penfluron (active ingredient) were prepared by dissolving in water. LC_{50} and LD_{50} of penfluron for *P. demoleus* for a 48 h period were calculated by plotting the progress of mortality and time of exposure to penfluron on log probit paper. Based on these lethal concentrations, 3 sublethal doses of penfluron were prepared and used for topical and oral experiment.

†Part of Ph.D thesis submitted to Madurai Kamaraj University by the first author.

For oral administration experiment, larvae were allowed to feed on penfluron treated leaves for one day and then untreated leaves were offered for the remaining larval period. For topical application experiment, larvae were treated with penfluron and they were allowed to feed on untreated leaves till the completion of larval period. For control experiment, larvae were fed on leaves without treatment of penfluron. Each experiment was replicated 5 times. The impact of penfluron was assessed based on conversion and utilization efficiencies of feeding on the host plant, *C. limonum*.

2.2 *Calculation of feeding efficiencies*

The following food conversion and utilization efficiencies were calculated as described by Waldbauer (1968) and Petrusewicz and MacFadyen (1970).

Relative consumption rate (RCR) = mg biomass eaten/g larval biomass/day.

Relative growth rate (RGR) = mg biomass gained/g larval biomass/day.

$$\text{Approximate digestibility (AD)} = \frac{\text{Assimilation (mg dry wt)}}{\text{Consumption (mg dry wt)}} \times 100.$$

$$\text{Efficiency of conversion of digested food (ECD)} = \frac{\text{Growth (mg dry wt)}}{\text{Assimilation (mg dry wt)}} \times 100.$$

3. Results

3.1 *Toxicity*

The efficacy of penfluron was investigated by oral and topical administrations. LD_{50} and LC_{50} values of penfluron for fifth instar larvae of *P. demoleus* were 9·8 and 7·6 μg/larva/48 h respectively. Of the two modes of application topical application produced 50% mortality at a very minimum dose of 7·6 μg.

Table 1. Effect of sublethal doses of penfluron on the food utilization in the fifth instar of *P. demoleus*.

Treatment (μg)	Consumption	Assimilation	Growth	Metabolization
Control	1825·26 ± 172·20	695·26 ± 59·00	169·87 ± 15·00	525·39 ± 51·14
Oral				
0·33	2658·25 ± 251·12	1079·25 ± 101·00	160·00 ± 14·70	919·25 ± 70·00
0·98	1941·85 ± 180·90	979·20 ± 89·00	136·04 ± 13·00	843·16 ± 81·40
1·96	1897·46 ± 169·70	779·85 ± 78·60	99·42 ± 8·40[b]	680·43 ± 62·70
Topical				
0·25	1965·14 ± 178·15[a]	895·28 ± 82·30	158·00 ± 14·90	737·28 ± 70·00
0·76	1860·20 ± 180·00	796·12 ± 772·60	130·60 ± 12·70	665·52 ± 58·60
1·52	1560·20 ± 145·20	705·12 ± 69·75	110·20 ± 10·00[b]	594·92 ± 55·45

All values are expressed in mg/individual. $\bar{X} \pm SD$.
[a] $P < 0.1$; [b] $P < 0.001$.

Table 2. Effect of sublethal doses of penfluron on the food utilization in fifth instar of *P. demoleus*.

Treatment (μg)	Relative consumption rate (RCR)	Relative growth rate (RGR)	Approximate digestibility (AD)	Growth efficiency (ECD)
Control	$392{\cdot}19 \pm 32{\cdot}15$	$36{\cdot}49 \pm 6{\cdot}10$	$38{\cdot}09 \pm 5{\cdot}00$	$24{\cdot}43 \pm 4{\cdot}23$
Oral				
0·33	$695{\cdot}88 \pm 67{\cdot}00$	$41{\cdot}88 \pm 3{\cdot}50$	$40{\cdot}60 \pm 3{\cdot}00$	$14{\cdot}83 \pm 2{\cdot}00$
0·98	$585{\cdot}28 \pm 56{\cdot}00$	$41{\cdot}04 \pm 4{\cdot}00$	$50{\cdot}43 \pm 5{\cdot}00^{a}$	$13{\cdot}89 \pm 1{\cdot}65$
1·96	$639{\cdot}74 \pm 61{\cdot}00$	$33{\cdot}52 \pm 2{\cdot}90$	$41{\cdot}09 \pm 6{\cdot}00$	$12{\cdot}75 \pm 1{\cdot}45^{a}$
Topical				
0·25	$560{\cdot}51 \pm 53{\cdot}00$	$45{\cdot}07 \pm 5{\cdot}00$	$45{\cdot}56 \pm 3{\cdot}00$	$17{\cdot}65 \pm 2{\cdot}95$
0·76	$583{\cdot}13 \pm 58{\cdot}10$	$40{\cdot}94 \pm 4{\cdot}00$	$42{\cdot}80 \pm 5{\cdot}40$	$16{\cdot}40 \pm 2{\cdot}20$
1·52	$456{\cdot}47 \pm 46{\cdot}14$	$32{\cdot}24 \pm 3{\cdot}75$	$45{\cdot}19 \pm 4{\cdot}80^{b}$	$15{\cdot}63 \pm 1{\cdot}25^{a}$

Rates are in mg/g/day and efficiencies in percentage. $\bar{X} \pm$ SD.
$^{a}P < 0{\cdot}001$; $^{b}P < 0{\cdot}01$.

3.2 *Food utilization*

The results on feeding budget of *P. demoleus* indicate a greater quantity of food consumption in larvae subjected to both oral and topical administration of penfluron and also an enhanced consumption rate (RCR), growth rate (RGR) and assimilation efficiency (AD). However, growth efficiency (ECD) of larvae in both the treatments showed a significant decline compared to that of control (tables 1 and 2). Larvae of *P. demoleus* either treated with or feeding on maximum sublethal doses of penfluron gained significantly lesser weights, 110 and 99 mg, compared to control larva which gained 170 mg (table 1). These differences are statistically significant as shown in the following regression equation:

Topical application:

$$\text{Growth} = 163{\cdot}97 - 36{\cdot}80 \text{ penfluron}$$
$$r = -0{\cdot}9806;\ P < 0{\cdot}1.$$

Oral administration:

$$\text{Growth} = 172{\cdot}25 - 37{\cdot}09 \text{ penfluron}$$
$$r = -0{\cdot}9988;\ P < 0{\cdot}05.$$

4. Discussion

4.1 *Toxicity*

The LD_{50} and LC_{50} values of 9·8 and 7·6 μg/larva/48 h showed that those doses of penfluron compound required to cause 50% mortality to an oligophagous pest, *P. demoleus* are considered to be very minimum when compared to those values recorded for a polyphagous pest, *Pseudoplusia includens* (43·9 μg) (Reed and Bass 1980) and for a monophagous pest, *Ergolis merione* (27·76 μg) (Chockalingam and Krishnan 1984). Such a comparative account on the efficacy of chitin synthesis inhibitor compounds delineates the point that *P. demoleus* is more sensitive to penfluron than to its parental compound diflubenzuron (Somasundaram and Chockalingam 1988).

4.2 *Food utilization*

An enhanced level of food consumption observed at sublethal quantity of penfluron administered to *P. demoleus* either through topical or oral method may be to ensure intake of more food energy for its utilization at a later time to meet the impending energy demands that are likely to be warranted by toxic stress. Reports on the efficacy of diflubenzuron on the feeding physiology of monophagous pest, *E. merione* and an oligophagous pest, *P. demoleus* seem to be in conformity with the present findings of enhanced food consumption (Chockalingam and Krishnan 1984; Somasundaram and Chockalingam 1988). Similar report of higher consumption of food has also been recorded in cabbage webworm, *Crocidalmia binotalis* fed on a growth regulator compound, azadirachtin (Fagoonee 1983). The efficiency of *P. demoleus* to assimilate the food substances increased as the dosage of penfluron applied on the insect or fed along with food became higher. Such an increase in assimilation efficiency (AD) has already been reported in *Achaea junta* feeding on insecticide treated leaves (Ramdev and Rao 1980). Working on the effect of diflubenzuron on the feeding physiology of *P. demoleus* a similar kind of finding of an increase in AD with increasing dosage of diflubenzuron compound was drawn (Somasundaram and Chockalingam 1988). And this sort of increase in assimilation efficiency might be due to retention of food material in the gut for longer time (Mattson 1980). This might be due to a feeble peristaltic mobility of the gut caused by the action of insecticide accumulation in the alimentary tract of insects (Price 1975). However, growth and growth efficiency are lesser in all treatments compared with control experiment. Hence, it is concluded that higher consumption generally leads to poor growth and growth efficiency (ECD). This means that efficacy of digestive and metabolic process suffers when a lot of food is ingested. At 1·96 and 1·52 μg penfluron administered through oral and topical methods, detoxification mechanisms reach a maximum and food utilization is hindered; this is reflected in poor growth and growth efficiency (ECD). Much metabolic energy is wasted in detoxification process; nearly about 85–87% of assimilated food was utilized on metabolic activity of *P. demoleus* indicating that *P. demoleus* could divert a large part of the assimilated energy to confront with toxic stress. Lepidopterans such as *E. merione* (Chockalingam and Krishnan 1984) and *Pericallia ricini* (Krishnan 1984) expended more energy to detoxify the toxicity when exposed to various doses of diflubenzuron.

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Diurnal rhythms and seasonal changes in the roosting behaviour of Indian myna *Acridotheres tristis* (Linnaeus)

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Abstract. The present study deals with the awakening and roosting behaviour of mynas. In the annual cycle of these mynas, 3 distinct phases were noticed—the pre-breeding season (November–March), breeding season (April–July) and post-breeding season (August–October). The various roosting activities of mynas such as the time of waking up and calls, the time of arrival and departure of the first bird and up to the last bird, communal noise and the time of final settlement at the communal roost show a systematic diurnal and seasonal changes in relation to the times of sunset and sunrise (i.e. the light and dark periods of a 24 h day). These changes were found to be consistent at all the roosts under observations and during all the years of the study. These changes seem to be governed by endogenous rhythms, which are slightly altered due to the endocrine secretions particularly in the breeding season and are also modified due to the adverse climatic conditions.

Keywords. Diurnal rhythms; seasonal changes; roosting; bird behaviours; Indian myna; *Acridotheres tristis*.

1. Introduction

Studies on roosting behaviour of birds have constituted a popular subject in ornithological research in the world for many years. Such studies on roosting behaviour of Indian mynas have been reported by some workers in India and in other countries (Hindwood 1948; Gadgil 1972; Counsilman 1974; Feare 1976; Greig-Smith 1982; Sengupta 1982). Indian myna *Acridotheres tristis* (Linnaeus) Sturnidae: Passeriformes is a familiar urbanised bird distributed over the entire Indian sub-continent. Mynas are sociable in their habits. They are generally seen in pairs or in small flocks during daytime. In all the seasons, they roost communally at night in groups of 100–10,000 birds, either independently or forming a mixed roost alongwith some other species of birds.

Apart from the detailed study by Counsilman (1974) there is very little information on awakening and roosting behaviour of Indian mynas. We present in this paper the results of our observations on the various aspects of roosting behaviour of Indian mynas at Pune.

2. Materials and methods

Altogether 19 communal roosts located in Pune (18° 30′ N and 73° 53′ E) were censused at various times during June 1973 to July 1981. These roosts were designated by definite numbers R-I to R-XIX for the convenience of recording the observations.

2.1 *Roosting behaviour*

At all these roosts, observations on the time of arrival of the first bird in the evening and that of all the successively arriving birds up to the last bird, movements near the roost and the time of final settlement at the roost were recorded. These observations were carried out once in each month from June 1973 to August 1976 at each roost during successive 5 min interval, roughly between 1645 and 1930 h.

The observations on the time of awakening, movements near the roost and the time of departure of the first bird and of all the successively departing birds up to the last bird were made in the morning during successive 5 min interval roughly between 0500 and 0730 h. These observations were carried out fortnightly at two communal roosts for a fixed period as follows: at R-IV from August 1975 to September 1976 and at roost R-III from October 1975 to September 1976.

These pre-roosting and post-roosting behavioural observations were repeated for confirmation at roost R-IV during August 1980–July 1981.

3. Results

During the study period at all the 19 roosts, the mynas have roosted in close proximity with human settlement on trees in all the seasons. The following 3 phases were observed in the annual cycle of Indian mynas—the pre-breeding season (November–March), breeding season (April–July) and post-breeding season (August–October).

3.1 *Awakening and departure activity*

After a period of rest, mynas slowly wake up in the morning. Generally, waking of mynas starts about 40–50 min before sunrise. At first, only a single myna (and occasionally two mynas) wake up and emits a low-pitched call (beginning of noise). After this first call, there is silence for about 2 to 3 min. Once again there is a call followed by silence for about 1 to 2 min. The call continues. This sequence is repeated till the silent period is reduced to only a few seconds. Within a short time of 10–15 min most of the mynas thus wake up and begin to make the loud communal noise. While making the communal noise, mynas undertake certain movements at the roosting trees such as hopping, jumping between the branches and pushing each other. As the noise increases these movements also increase. This noise continues for another 15–20 min after which it slowly decreases as the mynas gradually leave the roost.

Figure 1 indicates the monthly and seasonal changes in the awakening and departing activities of mynas at roost R-IV during 1975–76. Throughout the post-breeding season from August–October, the time of sunrise occurs successively later. In the pre-breeding season also it occurs successively later from November–January and then successively earlier till March. In the breeding season, the time of sunrise occurs successively earlier from April–June and then later in July. In all these seasons, the waking of the first myna (beginning of noise) was found to be changing according to the time of sunrise. In the month of May (breeding season), waking up of the first bird was earliest (67 min before sunrise) as compared to other months of the year. The time taken for awakening (i.e. the time interval between the beginning

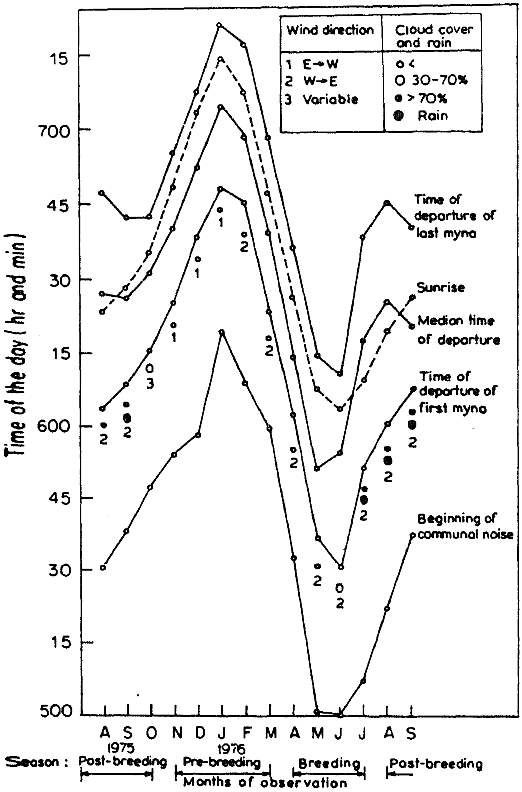

Figure 1. Monthly and seasonal changes in the time of awakening behaviour of mynas at roost R-IV during 1975–76.

: and median time of departure) of mynas at the roost was calculated, which monthly and seasonal variations (table 1).

time of departure of the first and the last myna changes according to the ' sunrise in all the seasons. The time of departure of the first and the last was always before and after the time of sunrise respectively. The duration n the time of sunrise and that of departure of the first and the last myna was :r not constant, but showed seasonal variations. In the month of June, the departure of the first myna from the roost was the earliest (33 min before ı and in the month of July, it was much later (18 min before sunrise). ly, in the month of December, the time of departure of last myna from the 'as earliest (4 min after sunrise) and in the month of July it was much later after sunrise).

seasonal changes in the median time of departure of mynas (i.e. the time at 50% of the total population of mynas have departed from the roost) were :nt with the changes in the time of sunrise. The median time of departure of was before sunrise except in the months of August 1975, July and August uring which it occurred 4, 8 and 6 min after sunrise respectively. In the of May, the median time of departure was the earliest (16 min before sunrise) pared to other months of the year.

Table 1. Monthly and seasonal changes in the awakening time and time span of departure of mynas at roost R-IV during 1975–76.

Season	Time taken for awakening (in min)	Total time span of departure (in min)
Post-breeding		
August (1975)	57	44
September	48	34
October	44	27
Pre-breeding		
November	46	30
December	54	29
January (1976)	45	33
February	50	32
March	40	35
Breeding		
April	42	34
May	51	38
June	55	40
July	70	47
Post-breeding		
August	63	45
September	43	33

The total time span of departure (i.e. the time between departure of the first and the last bird) was less in the pre-breeding season, medium in the post-breeding season and more in the breeding season. In the month of October the total time span of departure was lowest (27 min) and in the month of July it was maximum (47 min) as compared to other months of the year (table 1).

The time of departure in relation to time of sunrise and the number of mynas leaving the roost at R-IV has been indicated for each of the 3 seasons in figure 2. This figure shows that in the pre-breeding and breeding seasons more than 90% of the total population of mynas left the communal roost before sunrise. However, in July (end of the breeding season) and in August (beginning of the post-breeding season) only about 20–40% mynas left the roost before sunrise.

After leaving the roosting trees in the morning, mynas take to certain temporary halting places (trees, ground, etc.) near the roost before dispersing into feeding arena. Some differences were noticed in such movements of departing mynas in different seasons. It was observed that towards the end of post-breeding season, mynas started giving more preference to halt on ground and less preference to halt on trees after leaving the roost. This preference of coming down to ground gradually increased till the middle of the pre-breeding season. Thereafter, they gave less and less preference to halt on the ground in the breeding season and this continued till the beginning of the post-breeding season. Exactly reverse situation was noticed with respect to preference to halt on the trees.

3.2 *Arrival activity*

In the morning, mynas disperse into the feeding arena and spend the daytime in various activities such as feeding, nesting, making communication calls, resting and

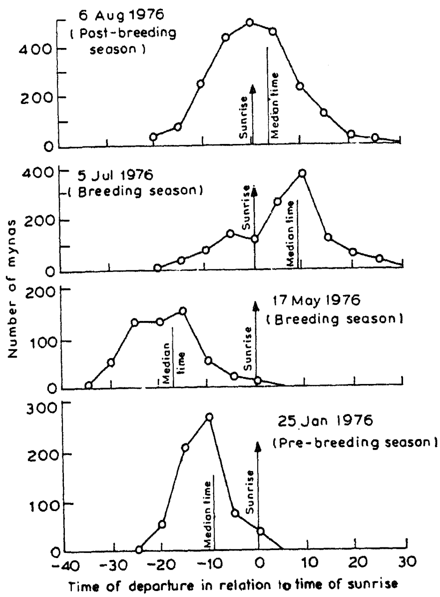

Figure 2. Seasonal variation in number of mynas departing before and after sunrise at roost R-IV.

preening. In the evening, mynas slowly start their return journey towards the communal roost. When they arrive at the roost, some of the mynas fly directly into the roosting trees. However, a majority of them take to certain temporary halting places (such as other trees, bushes, ground, etc.) in the immediate vicinity of the roost, before flying into roosting trees. A detailed study of these movements was undertaken at roost R-III between June 1973 and July 1974. It was found that the proportion of the above two categories of movements of arriving mynas varied from season to season. In the breeding season 37% mynas arrived at the roost from the feeding ground flew directly into the roosting trees. This number successively declined in the post-breeding and the pre-breeding seasons to 29 and 10% respectively.

It was observed that almost all the flocks of mynas made a big noise in the breeding season and till the middle of the post-breeding season while arriving in the vicinity of the roost. Thereafter, the number of flocks making noise was found to be decreasing and it was minimum in the pre-breeding season. This seasonal difference coincides with the noise made by mynas while departing from the roost in the morning.

The arrival activity of mynas was recorded at each communal roost in the evening during the entire study period. Figure 3 indicates the monthly and seasonal changes in the arrival activity of mynas during the year 1973–74. Throughout the post-breeding season from August to October, the time of sunset occurs successively earlier. In the pre-breeding season, it also occurs successively earlier till December and then successively later from January–March. In the breeding season, the time of sunset occurs successively later from April–June and then earlier in the month of July.

The time of arrival of the first and the last myna changes according to the time of

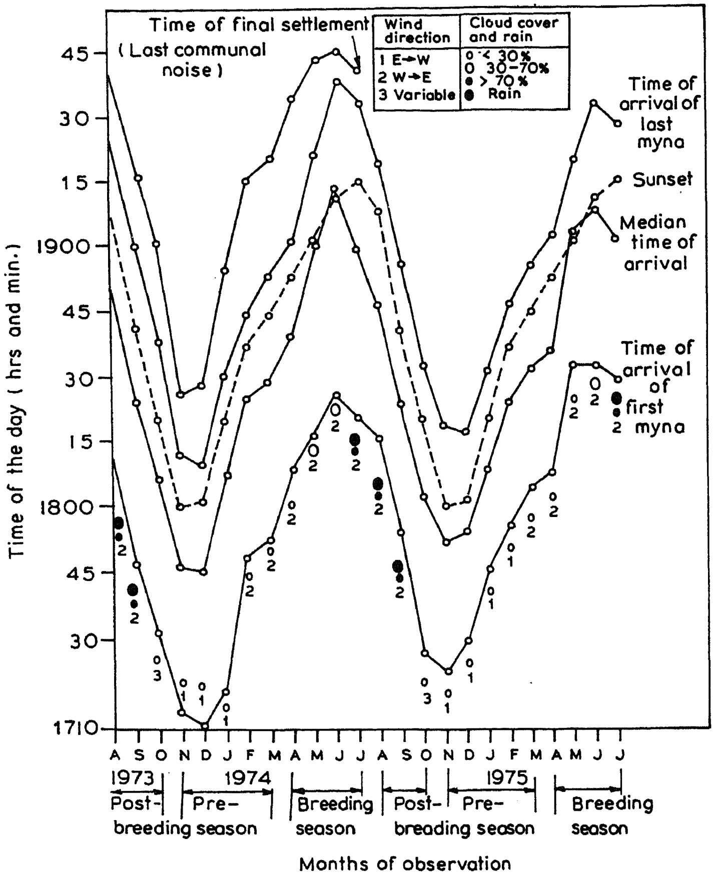

Figure 3. Monthly and seasonal changes in the time of arrival and final settlement of mynas during two successive years (mean of all roosts).

sunset in all the seasons. The time of arrival of the first and the last myna was always before and after the time of sunset respectively. The duration between the time of sunset and that of arrival of the first and the last myna was not constant, and showed seasonal variations. In the month of August 1973, the time of arrival of the first myna at the roost was the earliest (59 min before sunset) and in the month of April 1974 it was much later (44 min before sunset). Similarly, in the month of February 1974, the time of arrival of the last myna was found to be during sunset period (7 min after sunset) and it was much later (27 min after sunset) in June 1974, as compared to other months of the years 1973–74.

The seasonal changes in the median time of arrival of mynas (i.e. the time at which 50% of the total population of mynas have arrived at the roost) were almost consistent with the changes in the time of sunset. The median time of arrival of mynas was always before sunset except in the month of June 1974 during which it occurred later (2 min after sunset). In the month of August 1973 the median time of arrival was the earliest (19 min before sunset) as compared to other months of the year 1973–74.

The total time span of arrival (i.e. the time between arrival of the first and the last bird) was less in the pre-breeding season, medium in the breeding season and more

in the post-breeding season and it was maximum (75 min) in the month of July 1974 (table 2).

The time of arrival in relation to time of sunset and the number of mynas arriving at the roost R-VII has been indicated for each of 3 seasons in figure 4. This figure indicates that in the post-breeding and pre-breeding seasons more than 85% of the total population of mynas arrived at the communal roost before sunset.

Table 2. Monthly and seasonal changes in the time span of arrival and time for final settlement of mynas during 1973–74. (mean of all roosts).

Season	Total time span of arrival (in min)	Time taken for final settlement (in min)
Post-breeding		
August (1973)	74	48
September	73	52
October	68	54
Pre-breeding		
November	58	40
December	59	44
January (1974)	69	50
February	56	52
March	61	53
Breeding		
April	53	55
May	66	45
June	73	32
July	75	44

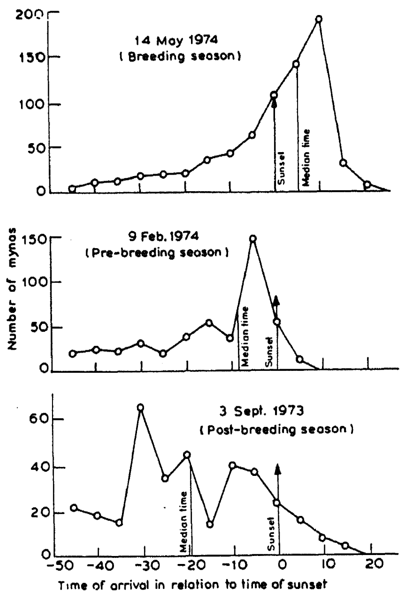

Figure 4. Seasonal variations in number of mynas arriving before and after sunset at roost R-VII.

However, during May (middle of the breeding season) only about 32% mynas arrived at the roost before sunset.

3.3 *Final settlement and communal sleep*

After arriving at the communal roost, mynas start making a low pitched noise. As more and more mynas join the roost, the noise gradually increases. Simultaneously, they undertake certain movements at the roosting trees such as hopping, jumping between the branches and pushing each other. When more than 50% of the mynas have joined the roost, such movements and also the communal noise reach the peak. This communal noise in the evening was much louder than that made in the morning before departing from the roost. At intervals, they suddenly stop making the communal noise and also the movements for few seconds. After this pause, they again continue to make the noise and movements. This sequence was repeated 5 to 6 times and the pause between these activities increases gradually. Finally, these activities stop altogether about 25–45 min after sunset, when mynas settled down at the roost and retire for the communal night's sleep or rest.

The time of last communal noise (time of final settlement) made by mynas was recorded at each communal roost late in the evening and it was found to be consistent with the changes in the time of sunset in all the seasons (figure 3). The time of last communal noise was earliest (26 min after sunset) in the month of November 1973 and it was much later (42 min after sunset) in the month of April and May 1974 as compared to other months of the year 1973–74. The time taken for final settlement (i.e. the time interval between the median time of arrival and the last communal noise) of mynas at the roost was calculated for the year 1973–74 which shows monthly and seasonal variations (table 2).

During the communal sleep at night, mynas occassionally wake up because of some known as well as unknown reasons. In few instances, they were found to be disturbed by an approaching owl (a bird of prey) and flying fox (a harmless mammal). After this temporary commotion, they again made the usual communal noise and the peculiar movements (described earlier) at the roost, before resuming their communal sleep. The night break-up was observed in every month of the year.

3.4 *Time spent at and outside the communal roost*

The total time spent at the communal roost (that is, the time interval between the median time of arrival of mynas in the evening and the median time of their departure in the morning) and the time spent outside the roost in the feeding arena during a 24 h day were estimated on the basis of departure and arrival activities of mynas at all roosts during the year 1975–76 (figure 5). The figure indicates that the proportion of time spent at and outside the communal roost changes monthly and seasonally. Generally, the time spent outside the roost was more or less the same (± 7 min) as the actual daytime period available between sunrise and sunset. The only exceptions were the months of July and August, when they spent 21 and 35 min less respectively in the feeding arena than the actual available daytime period. In the month of December (in the pre-breeding season), mynas spent maximum time (783 min) at the roost and minimum time (657 min) outside the roost. The reverse was the case in the month of June (in the breeding season), when

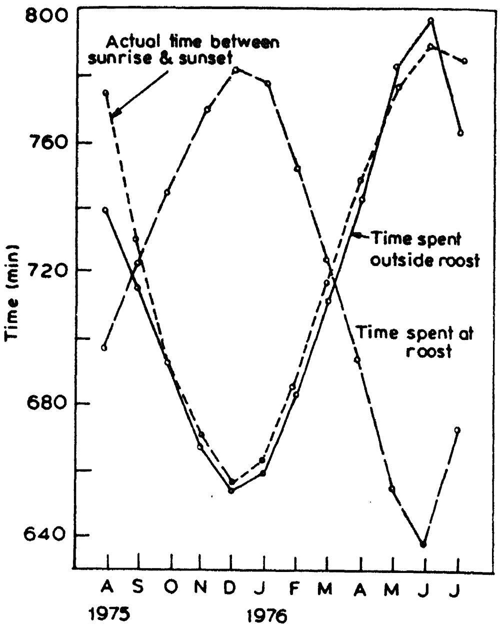

Figure 5. Time spent by mynas at and outside the roost during different months of 1975–76.

they spent minimum time (640 min) at the roost and maximum time (800 min) outside the roost.

3.5 *Confirmation of above observations in other years*

The observations carried out on the awakening and departure activities of mynas from the roost in the morning were also simultaneously studied at roost R-III during 1975–76. The monthly and seasonal changes were found to be identical to those at roost R-IV (figure 1). The observations carried out on the arrival activities of mynas at the roost in the evening were also studied further for two subsequent years, viz. 1974–75 (figure 3) and 1975–76. The pattern of monthly and seasonal changes were found to be substantially similar to the changes described for 1973–74 (figure 3). All the above morning and evening post- and pre-roosting behavioural observations were repeated at roost R-IV during August 1980–July 1981. It was confirmed that similar diurnal and seasonal trends exist in the roosting activities of mynas from year to year.

4. Discussion

Our studies clearly show that there are diurnal rhythms and seasonal changes in the awakening and roosting activities of mynas. These are influenced by environmental, physiological and behavioural factors.

Throughout the year, the morning departure (roost break-up) of mynas was shorter as compared to their evening arrival (assembly). This confirms the earlier

observation on the Indian myna by Counsilman (1974) and Greig–Smith (1982). Such behaviour has also been noticed in many other birds such as herons (Seibert 1951), starlings and magpies (Gyllin and Källander 1977a, b). This may be because of several facts, some of which are enumerated here. The departures are observed at their origin (Greig–Smith 1982). Further, there is presence of hunger stimulus in the morning (Swingland 1976). The reason may also lie in that the morning departure activity is under internal clock and social control. In general, there was longer time span of arrival of mynas in the evening. This could probably be related to their dispersion in the feeding arena as well as the availability and amount of food in the arena. Some abiotic factors such as wind speed, cloud cover may also be the reasons for longer time for assembly. Greig–Smith (1982) has stated that arrivals represent the end of flights from a variety of places.

The distribution of time spent by mynas during a 24 h day, at and outside the communal roost shows an yearly cycle. This cycle is closely related to the actual daytime available between sunrise and sunset. This has been reported in some other birds by Naik and Razack (1967); Gyllin and Källander (1976, 1977a, b) and also in common mynas *Acridotheres tristis* by Sengupta (1982).

The present observations on mynas reveal a definite relation between monthly and seasonal changes in the time of morning departure and evening arrival and the time of local sunrise and sunset respectively. Such relationship has also been pointed out in other birds by various workers; in house swift *Apus affinis* (Naik and Razack 1967), blue magpie *Cyanopica cyana* (Hosono 1967), cattle egret *Ardeola ibis* (Siegfried 1971), jackdaw *Corvus monedula* (Tast and Rassi 1973; Gyllin and Källander 1976), starling *Sturnus vulgaris* (Gyllin and Källander 1977a), magpie *Pica pica* (Gyllin and Källander 1977b) and also in common myna *Acridotheres tristis* (Counsilman 1974; Sengupta 1982).

The actual physical stimulus influencing the roosting activities in Indian mynas and in many other birds during the sunrise-sunset cycle may be that of light intensity. This has been reported by number of workers—see reviews of Siegfried (1971) and Counsilman (1974) as also contributions of Razack and Naik (1965); Hosono (1973); Tast and Rassi (1973); Daan (1976) and Swingland (1976). Aschoff (1967) has focussed attention on the fact that changing environmental condition alone do not cause the rhythmic changes from wakefulness to sleep, and that an endogenous rhythm is also present.

This endogenous activity rhythm is found to be slightly altered seasonally. Hormonal secretions in the body may bring about changes in several activities of mynas in the breeding season, particularly in the months of May and June. In these months, mynas behave differently. This view is supported by experimental evidence of Chaturvedi and Thapliyal (1980), who found that in common mynas *Acridotheres tristis* the adrenals and gonads were maximally active during May–June and were relatively inactive during the following months. Counsilman (1974) has also stated that during breeding season, when reproductive hormones of Indian mynas are most effective, they behave differently.

The endogenous activity rhythm is also slightly modified due to adverse climatic conditions such as heavy cloud cover and rains; particularly observed in the months of July–August. These climatic changes make most of the mynas to leave the roost after the time of sunrise and return to roost well before sunset. In many other birds such as blue magpie *Cyanopica cyana* (Hosono 1973), jackdaw *Corvus monedula*

(Tast and Rassi 1973; Gyllin and Källander 1976), starling *Sturnus vulgaris* (Gyllin and Källander 1977a), corn bunting *Emberiza calandra* (Gyllin 1967) and also in common myna *Acridotheres tristis* (Sengupta 1982), it has been reported that roosting takes place earlier in the evening with overcast, stormy, misty or rainy weather than in the evenings with clear weather. Dense cloud cover and rains during mid-day, however, do not bring about premature returning of mynas to the roost. This has also been pointed out by Counsilman (1974) and Sengupta (1982) in mynas. Gyllin (1967) has reported that in corn bunting *Emberiza calandra*, the internal clock or internal rhythm may well be of primary importance affecting the time of roosting and the light intensity may only be of secondary importance. This may perhaps be applicable to our studies on Indian mynas.

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Population dynamics of *Moina micrura* Kurz (Cladocera: Moinidae) inhabiting a eutrophic pond of Madurai (south India)*

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Abstract. Population density, composition, age structure and fecundity of *Moina micrura* have been studied in a eutrophic pond. The average clutch size of this species ranged from 1–4·4 eggs. The relationship between mean brood size and body length has been established. The volume of parthenogenetic eggs of this cladoceran ranges between 0·2 and 0·9 millions μ^3 and the adaptive significance of this has been discussed. The mean instantaneous birth rate which preceded maximum density of population resulted in a value of more than one.

Keywords. *Moina micrura*; population density; composition; age structure; fecundity; egg volume.

1. Introduction

Moina micrura, a member of the family Moinidae primarily inhabits astatic ponds and pools in tropical and subtropical regions. This species is highly adapted to survive frequent dry periods and propagate rapidly in newly formed ponds. The review of literature on zooplankton species of *Moina* shows that the information about the population dynamics of *M. micrura* from tropical Indian waters is far from complete. Hence, an attempt has been made to investigate its population density and composition, fecundity and age structure in natural habitats.

2. Study area

The present study was carried out in a seasonal shallow pond (figure 1) located in the Madura College campus at Madurai (Long: 78′8′ E; Lat: 9′56′ N), south India. The pond is exclusively rainfed with no outlets. On the southern and western sides, trees belonging to the species *Pongamia glabra*, *Azadiracta indica* and *Morinda tinctorea* encircle the margin of the pond. The trees give great stability to the margin of the pond and the litter in the form of dry fallen leaves allows enormous growth of zooplankton. There are no submerged aquatic plants although the microflora belonging to the family Cyanophyceae and the free floating macrophytes of the family Lemnaceae appear during certain periods.

3. Materials and methods

3.1 *Field data*

Zooplankton samples were collected at weekly intervals between August 26, 1976 to

*Part of the doctoral thesis.

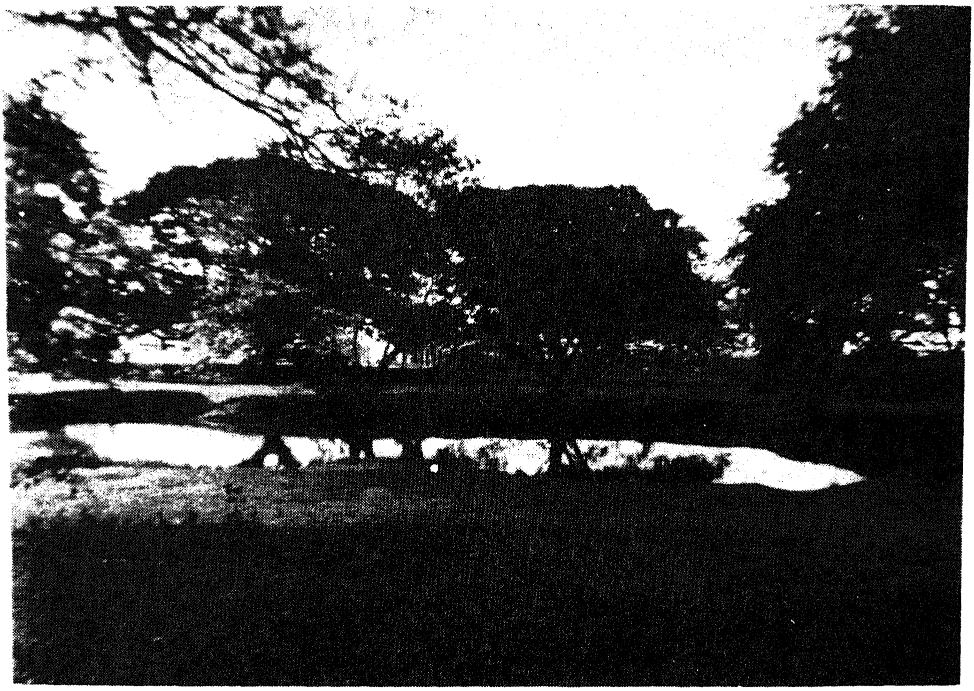

Figure 1. A view of the eutrophic pond.

July 15, 1977 from the surface and just above the bottom using a plastic bucket. The samples were concentrated by filtration through a bolting silk sieve of 120 μ mesh size. A total of 20 l of water was normally sieved. A total of 33 samples were obtained covering a period of 12 months. The samples collected on a given date were mixed and the concentrated plankton preserved in 4% formalin was used for the following biometric analyses.

(i) To identify the species and for counting population density and composition.
(ii) To identify the age structure.
(iii) To determine the fecundity.
(iv) To calculate birth and death rates.

3.2 *Species identification*

The recent revision of the genus *Moina* by Goulden (1968) was adopted for identifying the species as *micrura*. *Moina dubia* Gurney and Richard has been regarded as synonym of *M. micrura*.

3.3 *Population density and composition*

For counting the population densities 1/20 of the concentrate representing 20 l of pond water was used. The arithmetic mean densities of the species were expressed in numbers per litre. The population composition of *M. micrura* was

grouped into the following categories based on Green (1955).

(i) Immature females between 0·45 and 0·6 mm length.
(ii) Mature females without eggs (over 0·60 mm).
(iii) Females with parthenogenetic eggs or embryos.
(iv) Ephippial females.
(v) Males.

3.4 *Age structure*

Since *Moina* has no specific morphologic features to indicate age, it is difficult to analyse age structure from natural populations. However, it is possible to trace the same under laboratory conditions. Therefore different size classes were chosen after studying its ontogenic development under laboratory conditions. Thus 4 size groups viz. (i) $<0{\cdot}60$ mm, (ii) $>0{\cdot}60-<0{\cdot}79$ mm, (iii) $>0{\cdot}79-<0{\cdot}98$ mm and (iv) $>0{\cdot}98$ mm are recognised.

3.5 *Fecundity*

To determine the fecundity of the population from a mixed sample, the individuals were dissected out and eggs and embryos counted under a stereo binocular microscope. The mean number of eggs was calculated from the sample of 25 females with eggs in brood pouch. The number of eggs produced by a female varies with environmental conditions. The conditions which favour growth will also favour egg production (Elden 1943; Green 1954, 1955) and thus the two tend to fluctuate together. To test this, animals with eggs are measured from the crown to the posterior border of carapace using an ocular micrometer besides counting egg number. This measurement corresponds to the total length of Anderson (1932), the length of Edmondson (1955) and the body length of Lei and Clifford (1974).

3.6 *Egg volume*

The eggs from the parthenogenetic females were dissected out from the brood chambers in different sized individuals. The egg volume was calculated by using the formula $1/6\ gs^2$ where g is the maximum diameter and s is the minimum diameter (Green 1956; Lei and Clifford 1974).

3.7 *Birth rate, rate of population change and death rate*

The values obtained from counting the samples were analysed for birth rate, rate of population changes and death rate following Hall (1964) and George and Edwards (1974). This study enables to identify the population parameters of *M. micrura* in natural habitats and to some extent recognise the effect of environmental factors influencing the population. This method was first proposed by Edmondson (1960, 1968) for studying rotifer population dynamics and was applied to zooplanktonic populations by Hall (1964), Wright (1965), Cummins *et al* (1969), Dodson (1972) and George and Edwards (1974).

4. Results

The results of routine physical parameters of Madura College pond are given in figure 2. The day time oxygen concentrations in the pond water during the study period fluctuated around an average of 5 ppm. The pH fluctuation was between 7.2 and 9·5. Changes in the numerical standing crop of *M. micrura* are shown in figure 3. Figure 4 shows the seasonal variations in the mean length of the mature females and the number of eggs carried by them. The relationship between the mean brood size and the individual body length is represented in figure 5. Figure 6 illustrates the variations in the size distribution of *M. micrura*.

The percentage composition of the population of *M. micrura* are shown in table 1. Table 2 represents the diameters and volumes of parthenogenetic eggs of tropical and temperate Cladocera. Table 3 depicts the parameters of instantaneous birth rate, instantaneous rate of population increase and death rate of *M. micrura* population.

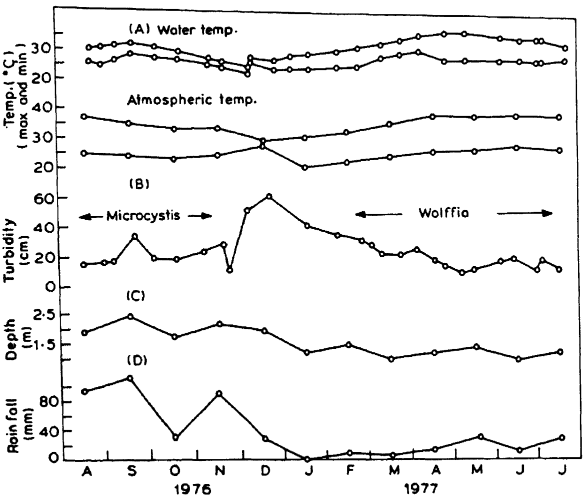

Figure 2. Seasonal variations in the physical parameters.

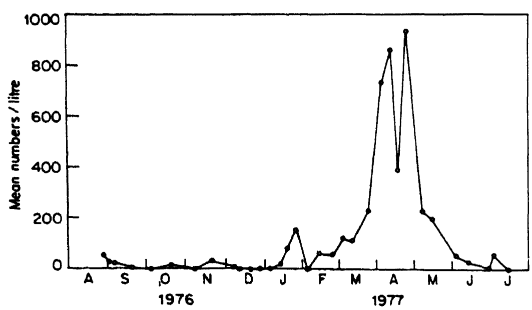

Figure 3. Changes in the standing crop of *M. micrura*.

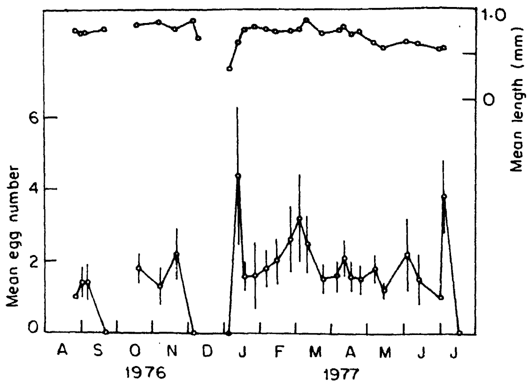

Figure 4. Seasonal variations in the mean length of mature females and mean number of eggs per brood.

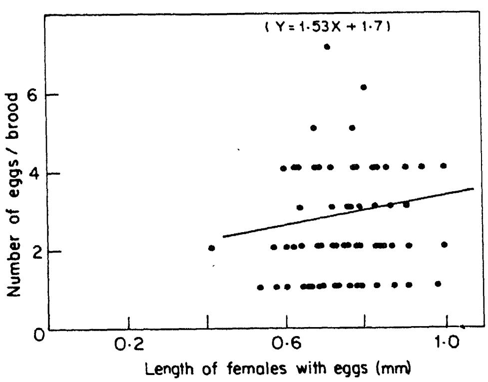

Figure 5. Relationship between the mean brood size and the body length in *M. micrura.*

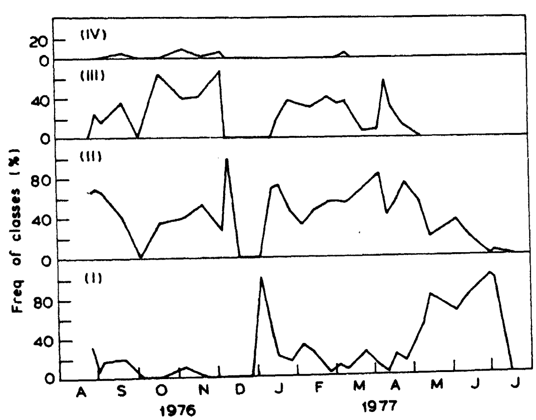

Figure 6. Seasonal changes in the size classes of *M. micrura.*

Table 1. Seasonal variation in the percentage composition of the population of *M. micrura* in Madura College pond.

Date	Immature females (%)	Females of mature size without eggs (%)	Females with partheno-genetic eggs (%)	Ephippial females (%)	Males (%)	No. of indivi-duals counted
1976						
26 Aug	32	64	4	0	0	50
29 Aug	8	48	44	0	0	50
5 Sept	18	54	8	20	0	50
19 Sept	20	65	0	15	0	20
3 Oct	0	0	0	0	0	0
18 Oct	0	36	64	0	0	25
6 Nov	10	50	40	0	0	10
19 Nov	0	13·3	86·6	0	0	30
5 Dec	0	100	0	0	0	15
9 Dec	0	0	0	0	0	0
18 Dec	0	0	0	0	0	0
25 Dec	0	0	0	0	0	0
1977						
3 Jan	100	0	0	0	0	5
12 Jan	40	24	36	0	0	25
17 Jan	18	42	26	4	10	50
25 Jan	16	44	40	0	0	50
4 Feb	33·3	0	66·6	0	0	6
14 Feb	23·3	33·6	40	0	0	30
25 Feb	4	28	68	0	0	50
3 Mar	10	26	64	0	0	50
10 Mar	6	46	20	18	10	50
23 Mar	21·81	38·18	29·09	1·81	9·09	55
4 Apr	8	26	48	14	4	50
11 Apr	2	34	56	8	0	50
17 Apr	20	28	52	0	0	50
24 Apr	14	22	62	0	2	50
6 May	48	14	38	0	0	50
14 May	70	10	20	0	0	50
3 Jun	48	4	44	4	0	25
12 Jun	60	0	40	0	0	5
29 Jun	70	0	30	0	0	10
3 Jul	84	0	16	0	0	25
15 Jul	0	0	0	0	0	0

5. Discussion

5.1 *Biology*

The life span of *M. micrura* is typical of many species of Cladocera. Reproduction occurs mostly by parthenogenesis. Early laboratory experiments on *Moina* species revealed that it has a life span of about two weeks and it produces approximately 61 young ones in 11 clutches (Murugan 1975). The parthenogenetic eggs are released into a dorsally located brood pouch where growth and development take place. The embryos are nourished through an unique structure

Table 2. Diameter and volume of parthenogenetic eggs of tropical (*M. micrura* and *S. acutirostratus*) and temperate (*S. vetulus*) Cladocera.

Date	Average water temp. (°C)	Size of individual (mm)	Number of eggs: Counted	Number of eggs: Measured	Mean egg diameter (μ): Largest (g)	Mean egg diameter (μ): Least (g)	Mean egg volume (millions μ^3)
M. micrura							
1976							
5 Sep	30·25	0·76	4	4	102·6	91·2	0·4
1977							
12 Jan	25·25	0·72	7	7	83·6	79·2	0·2
		0·60	4	4	91·2	87·4	0·3
		0·68	5	5	87·4	79·8	0·2
17 Jan	25·25	0·77	1	1	114·0	114·0	0·7
14 Feb	26·50	0·79	4	4	114·0	106·4	0·6
		0·83	2	2	127·5	106·4	0·7
		0·83	2	2	102·6	95·0	0·4
25 Feb		0·76	2	2	121·6	114·0	0·8
			2	2	136·8	114·0	0·9
3 Mar	29·25	0·91	2	2	125·4	117·8	0·9
S. acutirostratus							
1976							
18 Dec	26·00	2·38	15	14	239·0	224·0	6·2
25 Dec	24·50	2·50	25	12	245·0	204·0	5·3
	24·50	2.95	39	13	217·0	209·0	4·9
	24·50	3·86	70	25	208·0	195·0	4·1
1977							
17 Jan	25·25	2·32	7	7	224·0	212·0	5·2
24 Feb	26·50	2·54	27	14	247·0	245·0	7·7
25 Feb	27·00	2·38	26	11	256·0	246·0	8·1
3 Mar	29·75	2·31	13	6	212·0	204·0	4·6
10 Mar	29·75	2·27	13	8	207·0	194·0	4·0
S. vetulus							
1963							
30 Apr	15·00	—	—	60	246·0	218·0	6·1
14 May	14·00	—	—	40	248·0	228·0	6·7
10 Jun	21·00	—	—	70	245·0	216·0	6·0
13 Aug	18·00	—	—	50	252·0	223·0	6·6
28 Aug	16·00	—	—	40	255·0	233·0	7·3
25 Sep	14·50	—	—	12	262·0	235·0	7·6
16 Oct	12·00	—	—	20	272·0	247·0	8·6
6 Nov	10·00	—	—	30	270·0	243·0	8·3
27 Nov	7·50	—	—	55	270·0	250·0	8·8
4 Dec	6·00	—	—	20	271·0	244·0	8·4
11 Dec	3·50	—	—	30	287·0	259·0	10·1
20 Dec	0·50	—	—	50	280·0	254·0	9·5
S. vetulus (Greenland)							
1961							
28 Jul	11·00	—	—	22	308·0	273·0	12·0

Table 3. Population data for *M. micrura*.

	1	2	3	4	5	6	7	8
Date	N_o	N_A	E	L/D (day)	B	b	r	d
1976								
Aug 26	2700	1836	1·00	1	0·68	0·52	−0·17	0·69
29	1600	1472	1·41	1	1·29	0·83	−0·04	0·87
Sep 5	1200	984	1·41	1	1·15	0·77	−0·10	0·87
19	275	220	0·	1	0·80	0·59	−0·40	0·99
Oct 3	0	0	0	1	0	0	0	0
18	700	700	1·78	1	1·78	1·02	−0·10	1·12
Nov 6	100	90	1·33	1	1·19	0·79	+0·21	0·58
19	1675	1675	2·21	1	2·21	1·17	−0·06	1·23
Dec 5	625	625	0	1	1·00	0·70	−0·80	1·50
9	25	25	0	1	1·00	0·70	−0·35	1·05
18	0	0	0	1	0	0	0	0
25	0	0	0	1	0	0	0	0
1977								
Jan 3	50	0	0	1	0	0	+0·34	−0·34
12	1080	648	4·42	1	2·65	1·30	+0·26	1·04
17	3980	2865	1·66	1	1·19	0·79	+0·08	0·71
25	7775	6531	1·66	1	1·39	0·87	−0·40	1·27
Feb 4	130	86	1·75	1	1·16	0·77	+0·32	0·45
14	3225	2373	1·97	1	1·44	0·90	0	0·90
25	2915	2798	2·66	1	2·55	1·27	+0·12	1·15
Mar 3	6125	5512	3·20	1	2·88	1·36	−0·01	1·37
10	5550	4662	2·50	1	2·10	1·13	+0·06	1·07
23	11500	7944	1·50	1	1·03	0·71	+0·96	−0·25
Apr 4	36750	33810	1·63	1	1·49	0·92	+0·02	0·90
11	43000	42140	2·10	1	2·05	1·11	−0·13	1·24
17	19500	15600	1·58	1	1·26	0·82	+0·13	0·69
24	46800	40248	1·47	1	1·26	0·82	−0·12	0·94
May 6	11300	5876	1·76	1	0·91	0·65	−0·01	0·66
14	10000	3000	1·20	1	0·36	0·31	−0·06	0·37
Jun 3	2665	1385	2·20	1	1·14	0·76	−0·06	0·82
12	1500	600	1·50	1	0·60	0·47	0·08	0·55
29	333	99	1·00	1	0·30	0·26	+0·53	−0·27
Jul 3	2830	452	3·80	1	0·61	0·47	−0·66	1·13
15	0	0	0	1	0	0	0	0

1, Total population size/50 litres.
2, Total adults (adults/50 litres).
3, Mean number of eggs/brood.
4, Rate of growth or development of eggs.
5, Finite birth rate.
6, Instantaneous birth rate.
7, Observed instantaneous rate of population increase.
8, Estimated instantaneous mortality or death rate.

called "nahrboden" or "placenta" by the parthenogenetic adult females (Goulden 1968). The newly hatched young or neonates are born viviparously and resemble the adult in all respects except in size and the non-development of the ovaries. The neonates go through at least two pre-adult instars. The third instar is primiparous when the female lays her first batch of eggs into the brood pouch. The main sexual

dimorphism exhibited by the genus *Moina* lies in the antennules which are long and broadly curved in males adapted for clasping the females at the time of copulatory activities. In the female the antennules are cigarette shaped.

5.2 *Population density (standing crop) and composition*

The population of *M. micrura* has a single conspicuous peak in population density in April amounting to 936 units/l (figure 3). The brood size which is important in determining the total number of eggs in a population, had been increased gradually preceding the population maximum in *M. micrura*. The density was extremely low (few individuals/l) between August and December 1976. The possible reasons for low density may probably be explained by external factors viz. southwest (June–August) and northeast monsoons (October–December) which not only lowered the temperature (figure 2A) but also reduced the concentration of food in the medium. Further, the population phenomenon of low and discontinuous egg production (figure 4) resulted in decrease in density. Khalaf and Shihaf (1979) reported two population maxima in a year in *M. micrura*. The population composition of *M. micrura* has been analysed to determine when bisexual reproduction occurred and the extent to which females changed from parthenogenetic to gamogenesis (production of sexual eggs). Three periods of sexual reproduction, one in the beginning of September, second in the middle of January and the third in the second week of March 1977 occurred (table 1). The percentage of ephippial females varied between 15 and 20, 4 and 1·8 to 18. No males were found in September although the samples were thoroughly observed. The percentage of males recorded in the two latter periods amounted to 10. The repeated occurrence of ephippial females reflect the instability in the population structure of *M. micrura* which was extensively controlled by various factors such as food, temperature crowding etc. At this stage of our knowledge it would be premature to precisely interpret the causes for the switch over from parthenogenetic to gamogametic phase. However, it is interesting to observe that the occurrence of ephippial females (March–April) when the population was at its maximum, finds support from earlier observations of Berg (1931) and Green (1955), who also found that in *Daphnia* ephippial females appeared when the populations were densely crowded.

5.3 *Seasonal variations in egg production and body length*

The mean egg number is calculated by dividing the total number of eggs counted by the number of females with eggs. The changes in the brood size followed the general pattern of the mean length of females in *M. micrura* (figure 4). It has been already pointed out that the conditions which favour growth also favour egg production, the adult size and the brood size tend to fluctuate synchronously.

The mature females of this species formed 71·69% of the total population. The average clutch size ranged from 1–4·4 eggs. About 48·6% of the natural population has two eggs and 31·15% only a single egg. The percentage of females with 3 or 4 eggs was 18·84. Only 1·3% of the population has more than 5 eggs at any time. The maximum individual clutch size was 7 eggs. Almost similar clutch sizes were reported during an earlier laboratory investigation of this species (Murugan 1975).

5.4 *Mean brood size vs body length*

The number of eggs produced by a female is known to be influenced by a variety of factors which may be either intrinsic or extrinsic (Green 1956). The mean number of eggs potentially carried increases with the size of the female and the small body size naturally will restrict the egg laying capacity of the female (Green 1954, 1956). In the present study when the samples of parthenogenetically reproducing females of *M. micrura* was examined, there was found to be a low positive correlation ($r = 0{\cdot}13$) between the length and the number of eggs per brood. The regression equation was $Y = 1{\cdot}53 + 1{\cdot}7$ and the slope of the regression line $1{\cdot}53 \pm 2{\cdot}7$. It may be interesting to note that the egg number increases with the body length less rapidly since the body length of the females carrying eggs were in a narrow range which varied between 0·4 and 1 mm. This finding is in agreement with Green (1954, 1956) who already stated that small body size would restrict the egg laying capacity. It also appears that the space within the brood is an important limiting factor (Kerfoot 1974).

5.5 *Size distribution*

All size groups are well represented except class IV with two population size cycles. It may be assumed that the first generation of population which appeared in June took 6 months to reach the maximum percentage length of class III. Class IV individuals form a small percentage.

The development of a second size cycle of the population which appeared in January can be traced by successive length classes of II and III. The maximum population density in *M. micrura* in summer coincided with the length groups of classes II and III individuals which formed 84 and 56% respectively. The survival of class IV in this cycle was only 4%. It is sufficiently clear that the size distribution of *Moina* showed a marked variation in the natural habitats. The peaks of population density in this species coincided with the maximum proportion of mature individuals rather than immature forms.

5.6 *Egg volume*

The egg volume in *M. micrura* ranged from 0·2–0·9 millions μ^3 while that of the large sized *Simocephalus acutirostratus* (Murugan 1980) ranged from 4·1–8·1 millions μ^3 (table 2) which indicates the range of lowest and highest egg volumes in the two tropical species. Parallel comparison with forms living in temperate latitude indicated that *S. vetulus* (Green 1966) showed a range of 6–10·1 millions μ^3. The same species in Greenland, however, showed a mean egg volume of 12 millions μ^3. Thus there is an increase of about 1·5–3 times in the egg volume of more or less similar sized species with the change of latitude from tropics to temperate and arctic regions. This could be a positive effect of temperature (latitude) on egg volume. No such comparison could be made in the absence of ubiquitous of distribution and extensive studies on the species of *Moina* from cold, temperate and arctic regions. The adaptive significance of latitudinal differences in egg size has been described by Green (1966). A large egg gives rise to a larger neonate and there is a tendency within a species for the larger neonates to become mature at an earlier instar than do the

small neonate. In warm water the greater number of smaller eggs ensured a greater rate of population growth (Green 1966). It may be relevant to mention here that the smaller egg volume in *M. micrura* is likely to be an adaptation for rapid build up of population in the tropics. Green (1956) and Kerfoot (1974) indicated that the parthenogenetic eggs show size variation during different seasons. Hutchinson (1951) and Lack (1954) ascribed these changes of egg size as evolutionary responses that anticipated resource abundance i.e. when the food materials are in abundance the animal produced small eggs whereas the opposite was true when the food became scarce. The reserve food materials in the egg had to be stored in bigger eggs in view of the scarcity of food in the environment so that for a larger period the embryo can utilize the stored food in the food scarce environment.

5.7 *Population dynamics*

The observed instantaneous rates of population increase (r), calculated from successive pairs of population density data, and instantaneous birth rates (b), calculated on the basis of egg development time from the laboratory study are shown (table 3). These functions are applicable only to a population with stable age distribution and continuous reproduction. But this may also be applied for descriptive purposes to a population without a stable age distribution as has been previously used by Hall (1964), Edmondson (1968) and George and Edwards (1974). It is noted that potential rate of population increase (b) was higher in all months than the observed rate of population increase (r). The mean instantaneous birth rate value is more than 1 in March which preceded the population density maximum in April 1977. The increase in mean brood size from mid January to March 1977 (figure 4) and rise in water temperature (figure 2) are other causative factors for population maximum. The estimated instantaneous death rate (d) exceeding the instantaneous birth rate (b) were of considerable significance to account for the absence of population maxima of *M. micrura* in the early months of the study period. The population decline can be seen due to fall of birth rates and lesser number of eggs in the brood.

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Shell structure and shell strength in Cirripedes

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Abstract. Compressive and adhesive strengths of 7 barnacles endemic to Bombay shore were ascertained by using Instron universal testing machine. The adhesive strengths of balanid species settled on man-made materials like bakelite, asbestos, perspex, rubber, glass and teflon were determined. The adhesion on teflon was found to be the poorest. Comments are made on the compressive strengths of barnacles and their shell macro-structures. A need for the adoption of uniform method of the preparation of shell samples as well as of instrumental technique has been suggested for computing fresh data for the representative species.

Keywords. Mechanical strength; tropical barnacle; marine; man-made material.

1. Introduction

Attempts are currently made to evolve an antifouling technology that is free from use of chemical toxins like cuprous oxide and tributyl-tin oxide. Use of low energy surface materials or coatings is considered as one of the promising areas of research (Young and Crisp 1982). If this approach is to be proved effective, then the biological studies having relevance to the adhesion and bioadhesives of the fouling organisms should receive more attention. This aspect of study has relevance also to the maintenance of marine structures which are not easily approachable and need periodical manual or mechanical cleaning (Devoluy *et al* 1972).

Earlier reports on the assessment of compressive and adhesive strengths of barnacles have bearing on host-predator relationship (Qasim 1957; Palmer 1982) and on shell strength and wave action (Gubbay 1983; Barnes *et al* 1970; Murdock and Currey 1978). Some of the important publications describing the shell structure in acorn barnacles are by Cornwall (1956, 1958, 1959, 1960), Costlow (1956), Read (1960), Stubbings (1967), Ross (1970, 1971), Newman and Ross (1971), Bourget and Crisp (1975), Klepal and Barnes (1975) and Otway and Anderson (1985). Udhayakumar and Karande (1986) have reported adhesive strengths of balanid and chthamalid barnacles.

The present work was carried out with a view to adding some more information to the existing literature on barnacle adhesive and compressive strengths. Report on shell design and architecture of 8 barnacle species endemic to Bombay shore is communicated elsewhere for publication.

2. Materials and methods

In the present study, the methods adopted by Barnes *et al* (1970), Bourget (1977), Murdock and Currey (1978) and Gubbay (1983) were generally followed. Seven species of barnacles viz. *Balanus amphitrite amphitrite* (Darwin), *B. variegatus*

(Darwin), *B. amaryllis euamaryllis* (Broch), *Chthamalus malayensis* (Pilsbry), *Ch. withersi* (Pilsbry), *Tetraclitella karandei* (Ross) and *T. purpurascens* Wood settled on boulders were examined for the tension and compression strengths.

2.1 *Tension (adhesive) tests*

For recording the adhesive strengths, Instron model 1123 universal testing machine was used. The apparatus consisted of a strain gauge load cell secured to a moving cross-head. A stainless steel wire was glued to a solitary shell with the help of araldite resin. During the experiment the barnacle with its substrate was held securely in a vice and a hook of the embedded wire was interlocked with the wire hook held in the jaws fitted on the cross-head of the equipment. The experiments were carried out at a constant cross-head speed and the maximum load borne prior to catastrophic failure i.e. the detachment of the shell from the substratum, was recorded.

2.2 *Compression tests*

For compression testing i.e. loading from above over the shell, the same Instron equipment was used. The individual barnacle settled on a substratum was held in a vice with its opercular plates lying horizontal and the load applied on the shell by flat stainless steel cylinder secured to a moving load cell. The maximum load borne before shell failure by breakage was recorded.

3. **Results**

3.1 *Shell design and architecture*

There are no reports on macrostructure of the shells of Indian barnacles. Recently Udhayakumar (1988) examined shell sculpturing of the species endemic to Bombay shore. A short account of macrostructure of megabalanus *B. t. tintinnabulum* is given below. This will assist in acquaintance with the terminology used in shell architecture and shell strength described in the following sections.

In *B. t. tintinnabulum* the parietal plates at apical (figure 1A), middle (figure 1B) and at basal level (figure 1C) show lap, scarf and butt joints respectively.

The carinal margin of each radius having distinctive bipinnate teeth is received along the edge of the adjoining plate (figure 1D). It shows good interlocking with elaborately sculptured edge of the adjacent pariete (figure 1E). The radial margin is in firm attachment with the entire height of the adjoining plate. The sculpturing of alar-rostral margin (figure 1F) is not elaborate. Each pariete shows a row of longitudinal hollow canals (figure 1B). Each longitudinal septum of the pariete is elaborated into massive wedge shape pinnate process (figure 1C) that sits in hollow of the radial canal of the basal plate at the junction of the basal plate and parietes.

The secondary septae originating from the inner side of the outer lamina of parietes are simple spiny projections (figure 1C).

Calcareous basal shell plate is thick particularly along the circumference. The base is traversed by main radiating canals (figure 1C) and several smaller secondary canals. All these radiating canals are septate.

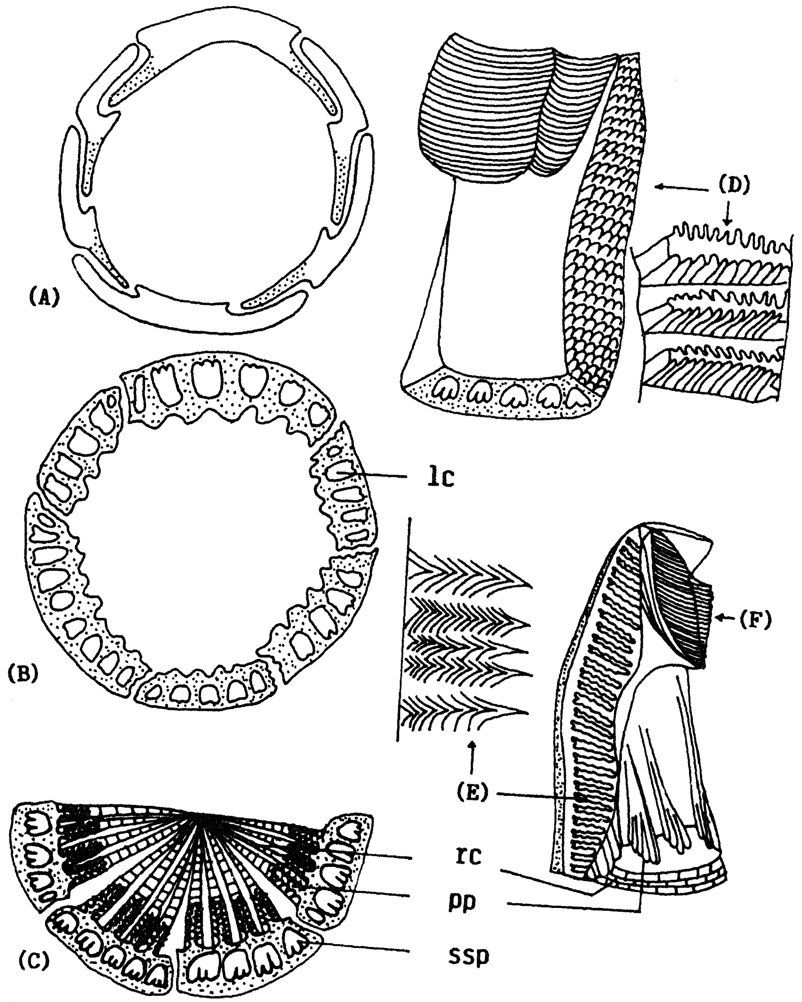

Figure 1. *B. t. tintinnabulum*, semidiagrammatic macrostructure of shell. **A–C.** Transverse sections of shell at apical (**A**), middle (**B**) and basal level (**C**). **D.** Carinal margin of radius. **E.** Groove along the edge of the paries of the carina. **F.** Ala-rostral margin.
(lc, Longitudinal canal; pp, pinnate process of longitudinal septa; rc, radial canal; ssp, secondary spinose processes or teeth.)

3.2 *Compression strengths of barnacles*

In this part of the study, 5 species viz. *B. a. amphitrite*, *B. variegatus* (calcareous base), *T. karandei*, *Ch. malayensis* and *Ch. withersi* (membranous base) were chosen. No observations could be made on *B. t. tintinnabulum* and on *B. a. euamaryllis* because of their scanty presence on boulders in the coastal waters of Bombay.

Figure 2 illustrates the regression lines drawn from the data obtained on the maximum load borne by 5 species under compression loads applied. Table 1 gives a comparison of the strengths of shells under compression in different species. It is observed from this table that the compressive strengths of 4 species, including both

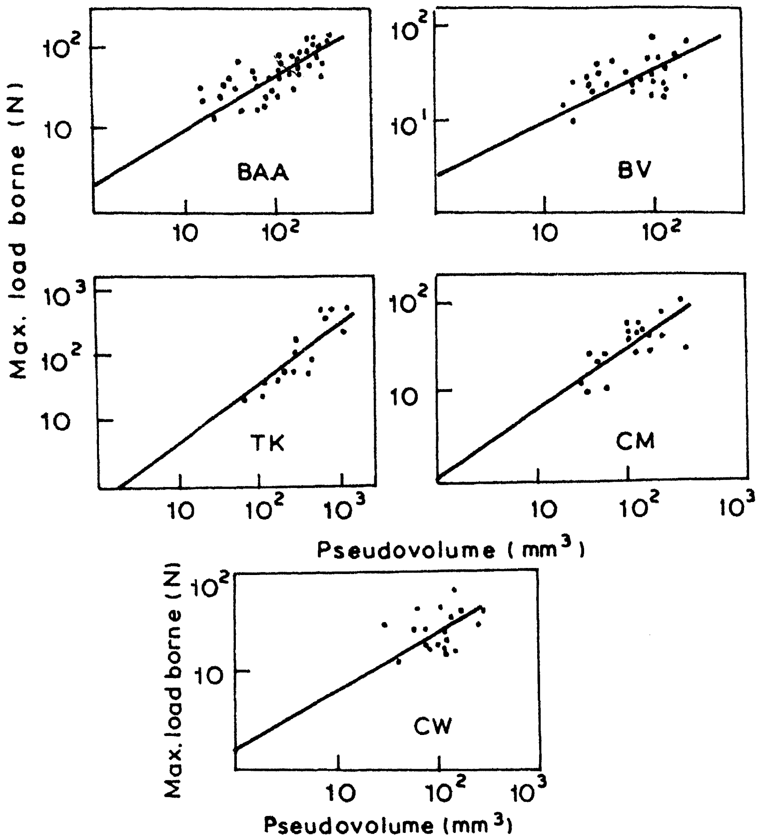

Figure 2. Compression load (N) required to bring about catastrophic failure of shells of solitary barnacles.
(BAA, *B. a. amphitrite*; BV, *B. variegatus*; TK, *T. karandei*; CM, *Ch. malayensis*; CW, *Ch. withersi*).

Table 1. A comparison of the shell strengths under compression of various species of barnacles.

Species	Pseudo volume (mm^3)	Maximum load borne (N) prior to shell failure: Solitary	Crowded
B. a. amphitrite	100	46·77	—
B. variegatus	100	36·47	—
T. karandei	100	39·81	101·62
Ch. malayensis	100	41·30	61·94
Ch. withersi	100	25·82	42·36

The maximum load values are taken from the regression lines fitted to the data.

calcareous base and membrane base, are comparable (between 36·47–46·77 N 100 mm^3). Only one chthamalid *Ch. withersi* shows poor compressive strength a compared to the others. A compressive strength of *Ch. malayensis* is very close t

that of *B. a. amphitrite* and is even more than that of *B. variegatus*. Again compressive strengths of calcareous base *B. variegatus* and membrane base *T. karandei* are comparable.

The compressive strengths of individual or a single *Ch. malayensis*, *Ch. withersi* and *T. karandei* growing in crowds were also recorded so as to ascertain if the support offered by the neighbouring individuals contributed to the enhancement of load bearing capacity of an individual member in the crowd. It is observed from table 1, that all these barnacles show a very notable increase in their capacity to bear load as compared to the individuals of the same species growing in isolation. *T. karandei*, *Ch. malayensis* and *Ch. withersi* show 155, 55 and 64% improvement respectively in their load bearing abilities in crowded settlements.

3.3 *Tensile (adhesion) strengths of barnacles*

Seven species of barnacles including *B. a. euamaryllis* were tested under tension for assessing their adhesive strengths on the rocky substratum they had attached. Adhesive strength of *B. variegatus* settled on bakelite surface was recorded since the specimens settled on rocky surface were not available in coastal waters. The results obtained are based on the examination of 30–60 individuals of each species tested.

It is observed from table 2 that the adhesive strength of *B. a. amphitrite*, amongst the species studied, is the highest ($1{\cdot}133 \times 10^8$ N m^{-2}), followed by that of *B. variegatus* and others. The adhesive strength of *T. purpurascens* has been found to be the least ($0{\cdot}12 \times 10^8$ Nm^{-2}). The adhesive strength of *B. a. amphitrite* is about 90% more than that of membrane base *T. purpurascens*. Generally barnacles having membranous bases show inferior adhesive strength than those with calcareous bases. However, a megabalanus, *B. a. euamaryllis* having a calcareous base also shows poor adhesive strength.

At a probability level of $P < 0{\cdot}10$, no significant difference in strengths of two balanids *B. a. amphitrite* and *B. variegatus* was noted. *B. a. amphitrite*, however showed significantly higher bonding strength as compared to all other species ($P < 0{\cdot}00005$). *B. a. euamaryllis* showed significantly more adhesive strength as compared to all membranous base chthamalids and tetraclitid but interestingly its

Table 2. Average adhesive strength (Nm^{-2}) of various species of barnacles settled on natural substrate.

Species	Average adhesive strength Nm^{-2} ($\times 10^8$)	Habitat
B. a. amphitrite	1·133	Intertidal
*B. variegatus**	0·860	Open sea
B. a. euamaryllis	0·268	Open sea
T. karandei	0·199	Neritic
T. karandei (on barnacle shell)	0·280	Neritic
Ch. withersi	0·149	Neritic
Ch. malayensis	0·131	Neritic
T. purpurascens	0·120	Neritic

**B. variegatus* settled on bakelite surface.

adhesion was found to be poorer than that of tetraclitilid *T. karandei* ($P<0.005$ to $P<0.00005$).

It is observed that the adhesive strength of *T. karandei* individuals settled on *B. a. euamaryllis* shell was superior to that of the individuals of the same species settled on rocky substrate.

3.4 *Adhesion on man-made substrates*

The adhesive strengths of two species viz. *B. a. amphitrite* and *B. variegatus*, very commonly encountered on various man-made materials, were examined. Two sets of coupons of various man-made materials were immersed in the seawater, each for periods of 35 and 85 days respectively to obtain individuals of varying ages for testing. It is observed from table 3, that the force required to dislodge both of these species is the highest for either glass or bakelite coupons, whereas it is the least in case of teflon coupons. The values obtained on rubber surface are not considered dependable for comparisons.

It has earlier been noted by Yule and Walker (1984) that a rough surface provides better opportunities for barnacle cyprids to achieve firm settlement. However, once having settled and grown to young adult, no particular benefit seems to be derived in securing a stronger bondage.

4. Discussion

4.1 *Compression strength*

Some of the easily recognisable shell features which contribute to its strength are the thickness of wall plates (Bourget 1977), plate porosity which arrests crack growth

Table 3. Average force (Nm^{-2}) required to dislodge two species of barnacles from various man-made surfaces.

	B. a. amphitrite		*B. variegatus*	
	35 days	85 days	35 days	85 days
Surface	$\times 10^8$ Nm^{-2}			
Teflon	0·0133 ± 0·0002	0·0313 ± 0·017	0·0115 ± 0·0003	—
Perspex smooth	0·35 ± 0·04	0·42 ± 0·03	—	0·15 ± 0·02
Perspex roughened	0·41 ± 0·01	0·178 ± 0·04	—	0·116 ± 0·016
Glass	0·67 ± 0·06	1·17 ± 0·14	0·60 ± 0·11	0·61 ± 0·04
Slate	0·59 ± 0·05	0·57 ± 0·02	0·48 ± 0·05	0·51 ± 0·03
Bakelite smooth	0·67 ± 0·06	0·94 ± 0·06	0·64 ± 0·04	0·86 ± 0·10
Bakelite roughened	0·51 ± 0·07	0·90 ± 0·07	0·59 ± 0·02	0·66 ± 0·06
Asbestos	0·51 ± 0·03	0·89 ± 0·03	0·51 ± 0·03	0·34 ± 0·02
Rubber	—	0·74 ± 0·05	0·61 ± 0·05	0·58 ± 0·03

(Barnes *et al* 1970), ridges or crenulations on plates (Murdock and Currey 1978), interlocking between parietes and base (Newman *et al* 1967; Murdock and Currey 1978) and the cuticle covering the shell plates (Parke and Moore 1935; Bonar 1936; Newman and Ross 1971). Besides these the strengthening of the plates by interspacing of organic tissue in the matrix of shell is also known to contribute to shell strength (Newman *et al* 1967).

Gubbay (1983) divided 7 temperate species into 3 groups viz. strong, intermediate and weak as regards their compressive strengths. He identified *Balanus balanus* having a compressive strength of 145 N/100 mm^3 as a strong barnacle. *B. crenatus* (calcareous base), *Eliminus modestus*, *Semibalanus balanoides* and *Ch. montagui* (all membrane base) having strengths between 35 and 57 N/100 mm^3 were categorised as intermediate ones. And *Verruca stroemia* (membrane base) having load bearing strength of 23 N/100 mm^3 was categorised as a weak barnacle. He observed that the species having membranous bases generally lacked the compressive strength.

Amongst the present Indian species, *B. a. amphitrite*, *B. variegatus* and *B. kondakovi* have comparable shell structures as well as interlocking sculpturing as that of *B. balanus* but have thinner wall plates and bases. These barnacles show less compressive strengths as compared to *B. balanus*. The importance of having thicker plates in addition to strong junctional interlockings therefore is obvious.

B. balanus which is shown to have a good compression strength, resembles *B. t. tintinnabulum* in its interlocking architecture between the individual parietes. Some variations, however, do exist. In *B. t. tintinnabulum* the sculpturing of the carinal margins of radii is more elaborate than that of *B. balanus* (Karande A A and Udhayakumar M, unpublished results). In both these species the longitudinal septae terminate into elaborate pinnate processes but in *B. t. tintinnabulum* secondary pinnate processes are absent. The parietes of *B. balanus* are not as heavy as *B. t. tintinnabulum*, but have heavily built ridges on inner and outer surfaces. *B. balanus* unlike *B. t. tintinnabulum* does not have radial canals in the basal plate and the depressions for receiving the pinnate processes of longitudinal septae are restricted to the periphery of the base. Comparison of the compressive strengths of these two species would therefore be worthwhile.

In the present study it was observed that the 4 members of 3 different genera *B. a. amphitrite*, *B. variegatus*, *T. karandei* and *Ch. malayensis* bore maximum load varying between 40–47 N/100 mm^3. No difference in strength between calcareous and membrane base barnacles was thus evident.

Despite a very elaborate sculpturing between the junctions, the presence of porous parietes as well as calcareous bases, *B. a. amphitrite* and *B. variegatus* show only ‘intermediate’ compressive strengths. On the other hand *Ch. malayensis* which lacks calcified base, porosity of parietes and pinnate ridges of the longitudinal septae, possesses load bearing capacity comparable to *B. a. amphitrite*. Its load bearing strength therefore is probably associated with heavily ribbed parietes. Membrane base *T. karandei* also has an ‘intermediate’ load strength and this can be attributed to its heavily ribbed surface, tubular parietes and with firm interlockings between adjoining parietal plates.

The above observations suggest that no one or two structural features contribute to shell strength. Species like *B. a. amphitrite* having all essential features for securing firm adherence amongst plates and between the latter and the base, may

fail in loading because of their thin parietal plates. On the other hand membrane base *Ch. malayensis* and *T. karandei* which lack good junctioning features may show a loading capacity comparable to *B. a. amphitrite* because of their heavily ribbed parietes. Barnes *et al* (1970) attributed loading strength of *Ch. stellatus* to its heavily ribbed parietes. They observe that 'the barnacle shell is a complex structure and its mechanical strength is dependent not only upon that of the individual parts but upon their structural relations and adhesion to one another'. Computation of what shell attributes of *Ch. malayensis* and *T. karandei* make these species as strong as *B. a. amphitrite* or *B. variegatus* would be a worthwhile study. Furthermore *T. karandei* has a distinctive sculpturing on the carinal edges of radii, it shows several short porous tubes in parietes, its walls are ribbed and has tubiferous finger-like extensions of radii around opercular opening (Ross 1971). Despite these shell features, its compressive strength is no better than that of *Ch. malayensis*.

Besides intrinsic shell characters, a crowding also enhances load bearing capacity of an individual barnacle as noted by Gubbay (1983) amongst *S. balanoides*. This was found to be true in 3 species examined in this study.

4.2 *Adhesive strength*

Barnacles secure adhesion with the help of continuously produced cementing material. Gubbay (1983) observed that the membrane base species secured more adhesion than the barnacles having calcified bases. In the present study 2 species viz. *B. a. amphitrite* and *B. variegatus* showed stronger adhesion than each of the 4 membrane base species. Interestingly a megabalanus *B. a. euamaryllis* showed the least adhesion as compared to other calcareous base barnacles. It is likely that as the basal plate of this balanid grows in size, the earlier laid cement loses its bonding strength. This might as well be true of the barnacles examined by Gubbay (1983). The age factor perhaps is relevant.

As reported earlier by several workers (Bultman *et al* 1984; Crisp and Walker 1987), the adhesion of barnacles on teflon surface was observed to be poorer as compared to the other surfaces. That a surface has some role to play in securing adhesion was also revealed by the fact that a better adhesion by individuals was achieved on barnacle shell than on rocky substratum.

Compressive and adhesive strengths of acorn barnacles have been determined by several workers adopting more or less an identical instrumental method. Minor variations, however, do exist in the preparation of the test samples as well as in the details of the measurement techniques. As a result of this, correlating the mechanical strength of barnacle with its shell architecture has become difficult. Therefore there is a need of adopting a uniform approach for recomputing fresh data for the representative species.

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Differentiation of male reproductive system in *Oryctes rhinoceros* (Coleoptera: Scarabaeidae)

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Abstract. The male reproductive system of adult *Oryctes rhinoceros* consists of a pair of testes with 6 follicles in each, connected to vasa deferentia by small vasa efferentia, a pair of accessory glands, a median ejaculatory duct and a chitinous intromittent organ. The differentiation of these structures in the successive instars is described in detail. In the first, second and early third (final) instar larvae the reproductive system is rudimentary consisting of a pair of testes (each testis is a rounded aggregate of 6 follicles held together in a connective tissue sheath), a pair of delicate ducts or the vasa deferentia connected to 'genital disc' at the mid-ventral aspect of the 9th abdominal sternite. A chitinous plate makes its appearance in the 'genital disc' of second instar and is discarded alongwith prepupal exuvium. In the pupa, each testicular follicle gets separated to independent disc shaped organ. The ejaculatory duct and accessory glands differentiate from the distal part of 'genital disc' whereas the proximal part gives rise to the intromittent organ. Definitive spermatogonia differentiate in the late third instar larval testis. Spermatocysts and meiotic figures are seen only in the pupal stage and spermiogenesis after adult emergence. Sperm bundles are noticed only in a 2–4-day old adult. Accessory glands release secretion in 10–14-day old adult. Gradual degeneration of testis and ducts is observed in the adults from the third month onwards.

Keywords. *Oryctes rhinoceros*; testicular follicles; vasa efferentia; vasa deferentia; ejaculatory duct; spermatids.

1. Introduction

Most of the literature on coleopteran reproductive system give scattered information dealing either with ontogeny of testis and reproductive duct or with the morphology and histology of adult reproductive system. Sharp and Muir (1912) and Muir (1918) described the anatomy, ontogeny and morphology of male genital tube in Coleoptera. Singh–Pruthi (1924) reported the post-embryonic development of male genital organs of *Tenebrio molitor* and Metcalfe (1932) studied the structure and development of reproductive system in 3 species of Coleoptera viz. *Sitodrepa panicea* L, *Gastroidea polygoni* L and *Anthonomus pomorum* L. Williams (1945) explained the anatomy of adult reproductive system in a number of coleopteran insects. The anatomy and histology of male reproductive system of adult *Tribolium castaneum* (Murad and Ahmad 1977) and *Platynotes punctatipennis* (Inamdar and Joshi 1986) have also been reported.

Virkki (1957, 1967) studied the structure of testis follicles and meiosis in scarabaeid beetles while Landa (1959) explained the spermatogenesis in adults of *Melolontha melolontha*. Menon (1969) studied the differentiation of testis and apical cells in *T. molitor*. Spermatogenesis in *Phyllophaga anxia* (Berberet and Helmes 1972) has been reported. A detailed study on the Verson's cells, aberrant spermatozoa and male accessory glands in *Hydrophilus olivaceus* has been carried out by Gundevia and Ramamurthy (1974, 1977) and Smrz (1978) studied the

spermatogenesis in *Agonum assimile*. Richard–Mercier (1979, 1981) reported the light and electronmicroscopic work on apical cells and evolution of germinal cells in *Leptinotarsa decemlineata*. *Oryctes rhinoceros*, a member of the family Scarabaeidae is a major pest of coconut palm. It was thought worthwhile to study the differentiation of gonads, reproductive tracts, accessory glands, intromittent organ and spermatogenesis because nothing is known about this system in this insect.

2. Materials and methods

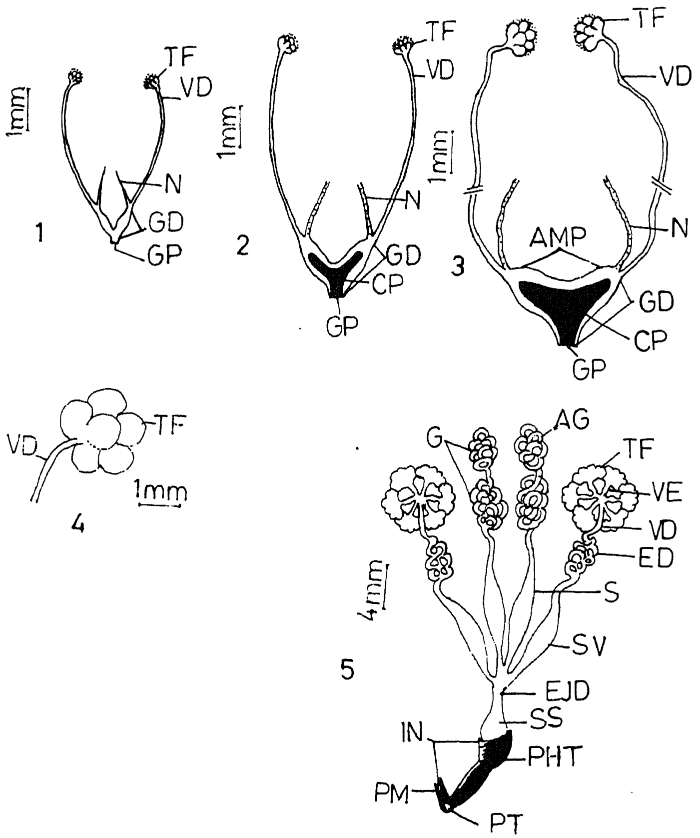
Third instar (final) larvae of *O. rhinoceros* were collected from the local manure pits and were reared individually in the laboratory on sterilized cowdung in small plastic containers (8 cm diameter × 9 cm height). Adults after one month were separated and kept in pairs in bigger containers (17 cm diameter × 30 cm height), half filled with dry cowdung and decaying leaves and fed on ripe banana slices or decaying plantain. The eggs which are laid 15–20 days after mating take 8–10 days to hatch. The first and second instar larvae moulted to successive stages after 12–22 days. The third instar larva takes 45–160 days before entering pupation. A short period of prepupal stage (6–8 days) which is marked by vigorous up and down movements of the larvae preceded pupal stage. Pupae moulted to adult after 16–18 days. Adults become reproductively mature after a month. Adults usually live up to 5 months, under laboratory conditions.

Etherized larvae of all stages, prepupae, pupae and adults were dissected and aqueous Bouin's fixative was added to fix and stain the ducts, gonads and 'genital disc' and they were rinsed in saline immediately. The reproductive system of different stages was thus traced. For histological studies, paraffin sections of testes and associated structures were fixed in aqueous Bouin's fluid for 12 h and stained in Heidenhain's haematoxylin eosin and in Mallory's triple stain. Measurements of testicular follicles and associated parts were taken using ocular micrometer.

3. Results

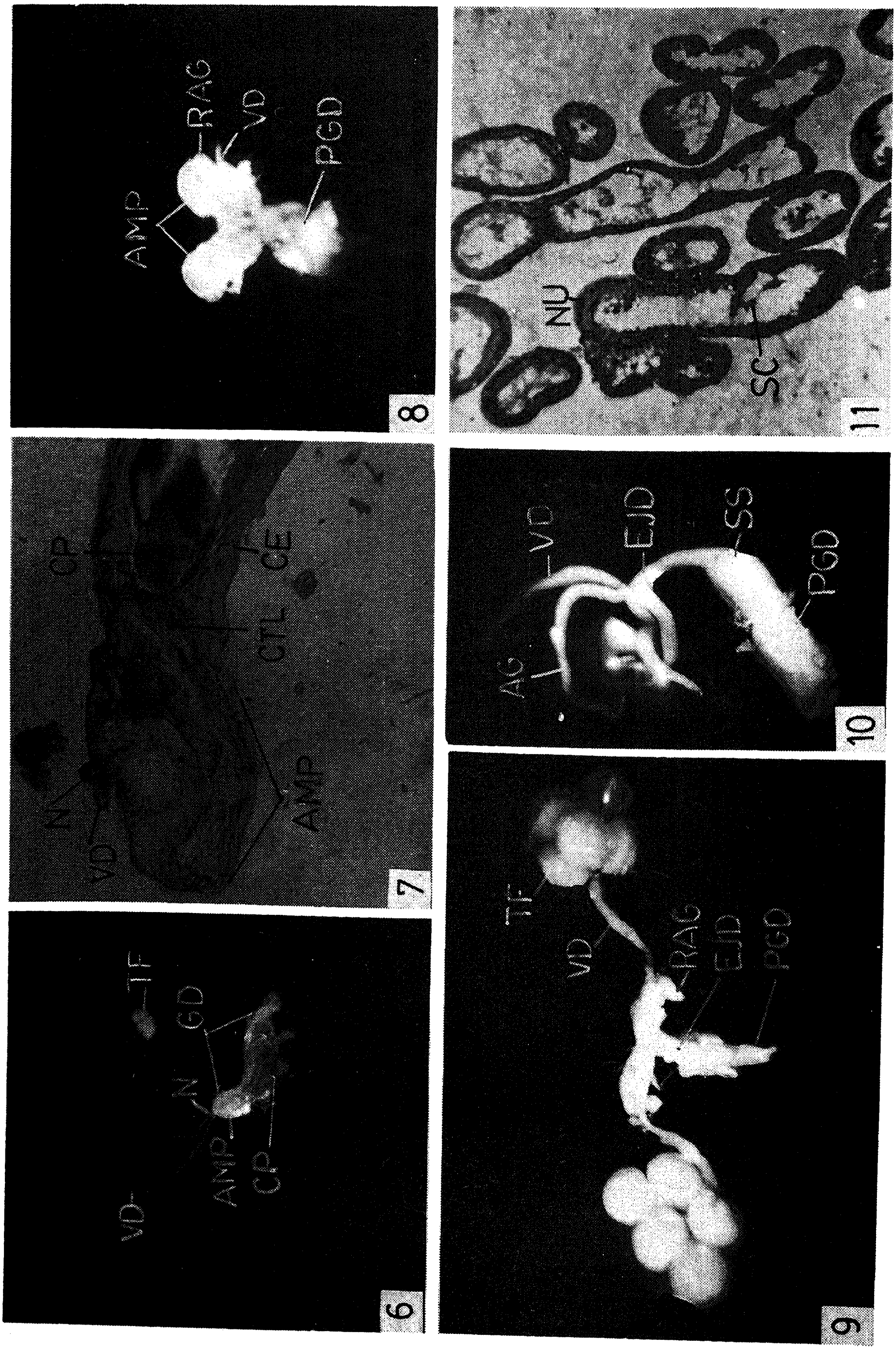
3.1 *Histomorphology of reproductive organs*

3.1a *Larva*: The reproductive system in the first (figure 1), second and early third (figure 2) instar male larvae appears rudimentary. In these a pair of testes with small round head are embedded in a thin layer of fat body on either side of the 7th abdominal segment. Each testis (210–460 μm diameter from first through early third instar) consists of an aggregate of 6 small round follicles, each being separated by thin strands of connective tissue sheath which also bind the follicles together. The vasa deferentia arising from the testes run postero-ventrally, joining with the 'genital disc' which is the imaginal disc of a part of internal genitalia and the whole of external genitalia, on the mid-ventral aspect of ninth sternite. The vas deferens is a delicate band of tissue, covered by connective tissue sheath ending in swollen ampullae. The duct in larvae is without lumen and it elongates from 3–7 mm in the first to early third instar larva. In the late third instar larvae these structures are more distinct (the testis is enlarged to 670–750 μm diameter, vas deferens 1·5–2 cm long and the 'genital disc' 2 mm in width, figures 3 and 6). The 'V' shaped 'genital



Figures 1–5. **1.** Reproductive system in a first instar male larva, genital disc without chitinous plate. **2.** Reproductive system in an early third instar larva. **3.** Reproductive system in late third instar larva. **4.** Testicular follicles in a one day old prepupa after attaining 'rosette' shape. **5.** Reproductive system of one month old male adult after developing seminal vesicle and epididymis.

' is composed of two parts; the anterior part being the swollen endings of the a deferentia and the posterior flattened structure is the ectodermal invagination he mid part (about 1 mm in width) of the 9th sternite into the body cavity. The terior part narrows towards the gonopore, distinguished by a chitinous trans-e scar on the 9th sternite. Besides the vas deferens, a nerve, which is a branch of 9th abdominal nerve also enters the ampulla just above the vas deferens. In ological preparations (figure 7) the 'genital disc' is lined up by an epithelium ing compactly arranged connective tissue layers inside. The epithelium at the on of the ampulla widens and forms a cup shaped depression into which the ; from vas deferens project. These cells are arranged just beneath the epithelium ning the lining of a blunt space inside the ampulla and this gives the appearance swelling to the ampulla. A chitinous plate makes its appearance in the 'genital ' of second instar larva and it becomes more distinct in the late third instar a. In histological sections it is seen attached to the connective tissue layers de the 'genital disc'. Male larvae (second and third) are thus identified by this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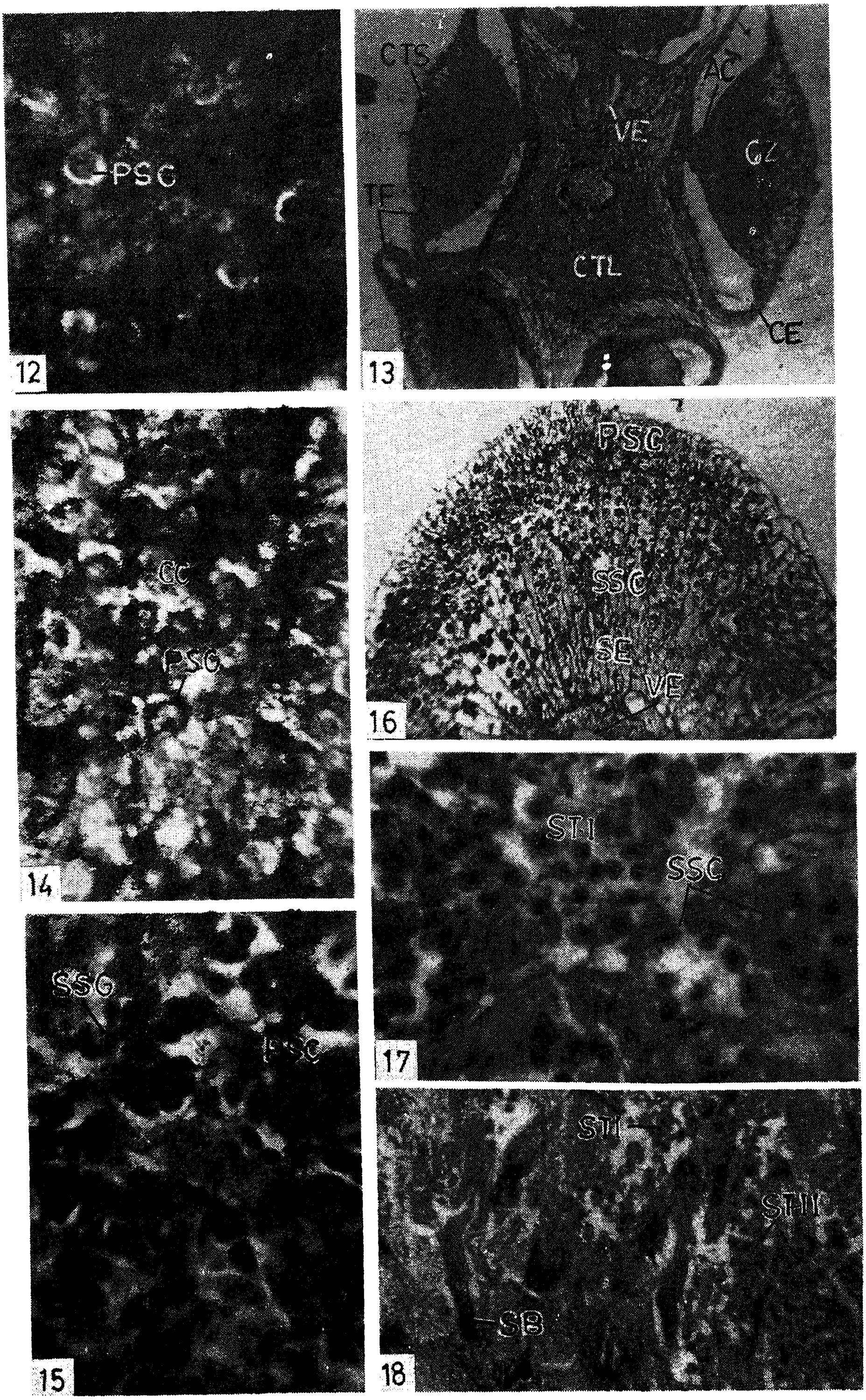
chitinous plate, visible through the ninth sternite. The first instar larvae are recognized by the gonopore, visible under a magnifying lens.

3.1b *Prepupa and pupa*: It is during pupal stage that the testicular follicles attain a 'rosette' shape (5 follicles arranged around a central follicle) and the testis as a whole is enlarged to 1 mm in diameter (figure 4). The chitinous plate in the 'genital disc' is expelled along with the prepupal exuvium by vigorous movements of the larva. Differentiation of each part of the male reproductive system occurs when the pupa is 1–8-day old. The connective tissue sheath holding the follicles together degenerates and each follicle becomes independent. The vasa efferentia develop by the extension of the follicular epithelium and become connected with the vas deferens. Each follicle is now 1 mm in diameter and appears as a disc. The vas deferens become short and thick measuring 5 mm in length. It develops the lumen inside. The apex of each ampulla now enlarges in size (figure 8) due to the proliferation of ectodermal cells from which a pair of evaginations develop into rudiments of the accessory glands. These rudiments grow and curve beneath the vasa deferentia (figure 9) and lengthen as a duct running parallel to vasa deferentia. Each duct, after reaching 3 mm in length develops fine tubules which later gets folded. This is the glandular part of the accessory gland while the proximal region becomes the storage organ in the adult. Meanwhile the part in between the ampullae elongates to a cylindrical tube, the ejaculatory duct. The bulbular nature of the ampulla is lost by now. In a 10-day old pupa the ejaculatory duct reaches about 3 mm in length (figure 10) and the posterior portion becomes swollen to form a muscular highly folded sac, the spermatophore sac. The portion just below the spermatophore sac covered with folded layers of connective tissue sheath becomes an organized intromittent organ towards adult moult.

3.1c *Adult*: The reproductive system of newly moulted adult is not different from that of a late pupa. However, each follicle gradually enlarges (2–3 mm diameter) its edges fringed as numerous tracheae ramify around them. In a month old adult (figure 5) each vas deferens becomes convoluted just behind the vasa efferentia forming epididymis, while the distal portion before joining the ejaculatory duct gets swollen forming the seminal vesicle due to the accumulation of spermatozoa. Similarly the duct of accessory gland before joining with ejaculatory duct expands to form the storage organ while the folded tubular portion becomes the glandular portion. The glandular portion is made up of a layer of columnar cells with distinct nuclei lined by a basement membrane (figure 11). Secretory granules become evident 6–8 days after adult emergence and release of secretory material is seen after 10–14 days.

Figures 6–11. **6.** Reproductive system of late third instar larva dissected and mounted in saline. Testicular follicles and duct of one side is only given ($\times 10$). **7.** Frontal section through one half of genital disc of late third instar larva ($\times 70$). **8.** One day old pupal genital disc, dissected and mounted in saline. See the enlargement of ampullae ($\times 7$). **9.** Six-day old pupal reproductive system dissected and mounted in saline. See the growth of accessory gland, only 5 testicular follicles are visible as one is hidden ($\times 7$). **10.** Ten-day old reproductive ducts dissected and mounted in saline ($\times 7$). **11** Transverse section through glandular folding of 10-day old adult accessory gland ($\times 70$).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The intromittent organ (figure 5) in *O. rhinoceros* is tubular consisting of a phallobase, an aedeagus and an endophallus. The phallobase is well developed forming a tube called phallotheca and it is the chitinous reddish brown structure with a wide anterior end covering the distal portion of spermatophore sac in which the spermatophore is formed, and a tapering posterior end with laterally placed paired parameres. The aedeagus is a sclerotised tube invaginated inside the phallotheca towards the distal end and is attached to the phallotheca by an apodeme, the aedeagal apodeme arising from the base of the aedeagus. The endophallus is the membranous, highly folded, eversible tube within the aedeagus which is a continuation of the ejaculatory duct and is protruded through the opening phallotreme, present at the distal end of the aedeagus, during copulation. At adult emergence, the endophallus is in the everted condition and is retracted into the aedeagus after a few hours. During copulation, the endophallus carrying the spermatophore, is pushed out through the phallotreme into the bursa of the female. The endophallus in the protruded condition forms the functional intromittent organ. Mating is observed after a month of emergence. From 3rd month onwards the testicular follicles and the ducts begin to degenerate.

3.2 *Histology of testis and spermatogenesis*

The testicular follicles of the first, second and early third instar larvae are composed of stem cells covered by connective tissue sheath. In the late third instar larva an epithelial layer of columnar cells beneath the connective tissue sheath is differentiated from the stem cells. Proliferation of the rest of the cells underneath this follicular epithelium is observed and large round cells with dusty chromatin appear among them surrounded by small cells, the cyst cells. They are the early stage of definitive spermatogonia or primary spermatogonia (figure 12). A group of lightly stained cells (apical cell complex) are seen in each follicle, but they are distinct only in prepupal testis. Considerable changes take place in the testicular follicles of

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Figures 12–18. 12. Definitive spermatogonia in the testicular follicle of late third instar larva (× 700). **13.** Transverse section through testis of 3-day old prepupa showing the arrangement of follicles and apical cells (× 80). **14.** Transverse section through 3-day old prepupal testicular follicle showing primary spermatogonia undergoing division (× 700). **15.** Ten h old pupal testis, transverse section showing secondary spermatogonia and primary spermatocytes (× 700). **16.** Transverse section through 16-day old pupal testicular follicle showing follicular septa and cysts arranged in the compartments (× 70). **17.** Transverse section through testicular follicle of 0-day old adult showing secondary spermatocysts and early spermatids (× 700). **18.** Transverse section through 2-day old adult testicular follicle showing spermatids and sperm bundles (× 300).

(*Abbreviations used*: AC, Apical cells; AG, accessory gland; AMP, ampulla(e); CC, cyst cells; CP, chitinous plate; CTL, connective tissue layers; CTS, connective tissue sheath; E, epithelium; ED, epididymis; EJD, ejaculatory duct; G, glandular part; GD, genital disc; GP, gonopore; GZ, germinal zone; IN, intromittent organ; N, nerve; NU, nucleus; PGD, proximal region of genital disc; PHT, phallotheca; PM, paramere; PSC, primary spermatocyte; PSG, primary spermatogonia; PT, phallotreme; RAG, rudiment of accessory gland; S, storage organ; SB, sperm bundle; SC, secretory content; SE, septa; SS, spermatophore sac; SSC, secondary spermatocyst; SSG, secondary spermatogonia; ST I, spermatids (early); ST II, elongating spermatids; SV, seminal vesicle; TF, testicular follicles; VD, vas deferens; VE, vas efferens).

prepupae as they assume a 'rosette' shape. In transverse sections (figure 13) the epithelium of each follicle appears to converge to a central region and the cells become flattened. The germinal cells of each follicle form a cup shaped zone being pushed towards the roof of each follicle, thus creating a space inside. The apical cell complex are now evident at the apex of each follicle near the spermatogonia. The germinal zone contains primary spermatogonia undergoing mitosis (figure 14). The epithelium gradually is confined only to the lower part of each follicle and the distal end narrows to form vas efferens and the vasa efferentia are kept together by connective tissue sheath and become free only at pupal stage. In a 6-day old prepupa, secondary spermatogonia characterized by dark chromatin, become evident in the spermatogonial cysts. Primary spermatocytes are observed in a 10 h old pupa as recognized by their enlarged size with dark chromatin (figure 15). Concurrent with the development of primary spermatocytes, the follicles also increase in size. Each follicle develops septa by the ingrowth of thin strands from the connective tissue sheath covering each follicle, extending towards the beginning of vas efferens. These intra-follicular septa divide the follicle into a number of compartments. The primary spermatocytes now migrate into these compartments and undergo meiosis and thus a maturation zone is developed. In a transverse section through 16-day old pupal testicular follicle (figure 16) primary spermatocytes are seen in the periphery of follicle and secondary spermatocytes towards the centre, these being arranged in the compartments. In the early pupa, the septa are not apparent in the germinal zone. However, in the late pupa as the germinal zone is reduced, septa are observed throughout the follicles. Meiotic figures are seen in the pupal stage, initiating from the 2-day old pupa. The testicular follicles of newly moulted adults are occupied by secondary spermatocytes and early spermatids (figure 17). In a 2–4-day old adult testis, spermatids of various stages and sperm bundles are observed (figure 18). In the adults, spermatogonia and meiotic figures are rarely seen. The testicular follicle of 2-month old adult is almost empty due to the release of spermatozoa starting from 8–10 days of emergence. Gradually the testicular follicles regress in size and degenerate.

4. Discussion

The male reproductive organs in the first, second and early third instar larvae of *O. rhinoceros* are rudimentary. However, these structures become distinct only in the late third instar larva, as reported for *T. molitor* (Menon 1969) and *P. anxia* (Berberet and Helmes 1972). However, in some Coleoptera these structures became apparent either at prepupal or pupal stages (Metcalfe 1932; Elliott 1964). It is interesting to note that in *L. decemlineata* (Richard–Mercier 1979) the spermatogenesis was over by larval stage itself.

The vasa deferentia are the only genital duct found during larval stages of *O. rhinoceros*. The original duct (mesodermal) is maintained with some structural changes taking place at pupal stage. According to Muir (1918) and Singh–Pruthi (1924) vas deferens in Coleoptera is a secondary structure and is ectodermal. However, Metcalfe (1932) gave evidence that vas deferens in Coleoptera is an outgrowth of testis and is mesodermal.

The 'genital disc' mentioned in the present study is comparable to the imaginal disc from which a part of internal genitalia and the whole of external genitalia

differentiate. This structure has been referred to as 'Herold's organ' (Verson and Bisson 1895); 'genital pocket' (Singh–Pruthi 1924); 'primary invagination of genital organ' (Metcalfe 1932); 'primary phallic organ' (Snodgrass 1935) and 'genital disc' (Whitten 1968). According to Reinecke *et al* (1983) Herold's organ in Lepidoptera has become established as a term applied for ectodermal invagination forming external genitalia and the inter part but the ampullae are excluded. As ampullae also contribute to the internal genital organ, 'genital disc' includes both ampullae and Herold's organ. Hence the term 'genital disc' seems to be more appropriate in *O. rhinoceros*.

There is only one pair of accessory gland in *O. rhinoceros* and they are ectodermal in origin (ectadenes) and vermiform. Accessory glands of similar nature had been reported for *P. anxia* (Berberet and Helmes 1972). In *H. olivaceus* (Gundevia and Ramamurthy 1977) two pairs of accessory glands of mesodermal origin are seen and the inner pair of glands are tubular showing almost similar structure as that of *O. rhinoceros*. Accessory glands of mesodermal and ectodermal origin together exist in *T. molitor* (Happ and Happ 1982) and *Platynotes punctatipennis* (Inamdar and Joshi 1986).

A median ejaculatory duct is present in *O. rhinoceros* and the posterior portion is swollen to form a sac like structure comparable to the spermatophore sac mentioned by Chapman (1982) and Davey (1984). According to Berberet and Helmes (1972) it is the erection fluid pump. Paired ejaculatory ducts are also seen in some Coleoptera (Metcalfe 1932). The intromittent organ in *O. rhinoceros* shows the typical structure described for Coleoptera by Snodgrass (1935). The phallobase is produced into a phallotheca as described by Davey (1984). The aedeagus and endophallus are invaginated inside the well developed phallotheca. Almost similar structure has been reported for *P. anxia* (Berberet and Helmes 1972).

Spermatogenesis in *O. rhinoceros* is initiated at the late larval instar as observed in *T. molitor* (Menon 1969). According to Virkki (1967) spermatogenesis in many melolonthines commenced in the pupal stage and is completed before adult emergence. In *O. rhinoceros* meiotic figures are seen in the pupal stage but sperm bundles are observed after adult emergence. A comparable condition is reported in *A. assimile* (Smrz 1978). However in *L. decemlineata* spermatogenesis is completed in the larval stage itself (Richard–Mercier 1979). Septum formation and subsequent division of each follicle into a number of compartments as observed in *O. rhinoceros*, is a characteristic feature of many coleopteran (Virkki 1957). According to this author, these septa assist in supplying nutrition and oxygen to the differentiating cysts. Apical cell complex are present in *O. rhinoceros*. They are seen in close association with spermatogonia. According to Gundevia and Ramamurthy (1974) Verson's cells encircle apical cell and are nutritive in function. The ultrastructural studies on apical cell (Menon 1969; Richard–Mercier 1981) also show that they are nutritive in function. Apical cell is not observed in *Tribolium castaneum* (Murad and Ahmad 1977) and in *P. punctatipennis* (Inamdar and Joshi 1986).

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Sclerotization of the periostracum of the marine bivalve *Perna viridis* (Linnaeus)

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Abstract. The enzyme phenoloxidase from the periostracum of the bivalve *Perna viridis* (Linnaeus 1758) was extracted and the substrate specificity was studied spectrophotometrically. The enzyme is solubilised by sodium dodecyl sulphate and is activated by trypsin. It shows high activity with the phenolic substrates pyrocatechol, dopamine (3–4 dihydroxyphenylethylamine) and dopa (3–4 dihydroxyphenylalanine). As the enzyme shows catalytic activity with many substrates, it may exist in a multiple form. A qualitative analysis of the phenols occurring in the mantle reveals the presence of dopa and dopamine which may play a role in the tanning of the periostracum. The nature and properties of the enzyme phenoloxidase from the periostracum of *Perna viridis* differ from that occurring in its byssal gland.

Keywords. *Perna viridis*; periostracum; sclerotization; phenoloxidase; mantle; phenols.

1. Introduction

In view of their ecological and economic importance, mussels as a group continue to receive attention in diverse areas of research. One such area is the stabilization of structural proteins. In bivalves scleroproteins are reported to occur as constituents of the periostracum—a thin fibrous layer covering the outer surface of the molluscan shell (Trueman 1950; Brown 1952). The secretion and extrusion of new periostracum from the periostracal groove of the mantle margin is accompanied by the rapid insolubilization, hardening and darkening of the extra-cellular material and this is effected by employing quinone to form quinone-tanned protein (Wilbur 1972). This process of sclerotization presumably gives the structure durability and chemical resistance (Wilbur 1964, 1972; Saleuddin and Petit 1983). Further the quinone tanning of the periostracum has been postulated to be an essential prerequisite for an orderly deposition of calcium carbonate crystals (Beedham and Trueman 1968; Taylor and Kennedy 1969; Petit *et al* 1980).

Among the precursors that go to form the periostracum, the enzyme phenoloxidase occupies a central position as in insects. It is of interest to note that the phenoloxidase from the different regions of an insect, namely blood, cuticle and oothecal wall differs markedly in its nature and substrate preference (Cottrell 1964; Hackman 1971; Brunet 1980). The occurrence of a phenoloxidase in the byssal gland of the bivalve *Perna viridis* (Linnaeus 1758) besides the periostracal phenoloxidase, prompted this study to assess the difference, if any, between these two enzymes (Bharathi 1982; Bharathi and Ramalingam 1983). The nature of the enzyme phenoloxidase (EC 1·14·18·1) from the periostracum of *P. viridis* and its behaviour to substrates have been studied. Further the phenols from the mantle have been extracted and identified to determine whether a correlation exists between the nature of phenols occurring *in situ* and the behaviour of the enzyme to substrates.

2. Materials and methods

Green mussel *P. viridis* was collected from the shore opposite to the University Buildings, Madras. The periostracum was removed by running a sharp scalpel parallel to the shell margin. Approximately 3 g weight of the periostracum was collected from 30 animals.

2.1 *Enzyme preparation and assay*

The enzyme was prepared and assayed following the method of Waite and Wilbur (1976). The enzyme activity was measured spectrophotometrically in a Unicam Sp 800 spectrophotometer at 30°C. The increase in the absorbance was recorded every minute immediately after the addition of the enzyme preparation to a mixture of 1 ml of Tris HCl buffer pH 7·4, and 2 ml of 0·01 M substrate in Tris HCl buffer taken in the sample cuvette. The protein content of the sample was estimated following the procedure of Lowry *et al* (1951). The results are expressed as Δ absorbance mg protein^{-1} min^{-1}.

2.2 *Substrate specificity*

The following chemicals in Tris HCl buffer were used as substrates: 0·01 M L-tyrosine, tyramine, dopa, dopamine, pyrocatechol, protocatechuic acid and hydroquinone (Sigma Chemicals Co., USA). The enzyme-catalysed oxidation of the above catecholic substrates was studied by monitoring the formation of quinones at their wave length of maximal absorption (Waite and Wilbur 1976; Waite 1976). The molar extinction coefficients of the quinones are listed in table 1. Using the molar extinction coefficients the values of Δ absorbance mg protein^{-1} min^{-1} were converted into μmol of substrate oxidized per min (Waite and Wilbur 1976).

2.3 *Extraction of phenols*

The phenolic compounds were extracted from the mantle of *P. viridis* following the method outlined by Andersen (1980). The extracted phenols were characterised by thin layer chromatography and identified (Andersen 1980; Sekeris and Herrlich 1966).

3. Results

The steps in solubilization and activation of enzyme phenoloxidase from the periostracum are given in table 2. Homogenization of the periostracum in aqueous buffer 0·1 M Tris HCl, pH 7·4 liberates some measurable activity into the supernatant. Treatment of the pellet with sodium dodecyl sulphate (SDS) completely solubilises the periostracal phenoloxidase which is still further activated by the addition of trypsin. Keeping the μmol of dopa oxidized by the enzyme/mg protein/min as 1, the relative velocities of the enzyme activity with other substrates have been calculated (table 3). The substrate specificity experiments reveal that the enzyme shows maximum activity with the substrate pyrocatechol and secondly with

Table 1. Extinction coefficients of the oxidation products of the phenolic substrates used.

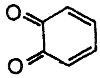
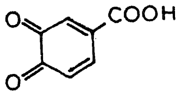
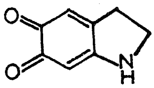
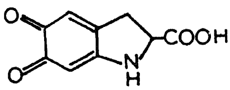
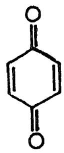
Substrates	Quinone produced		λ_{max} (nm)	E $M^{-1}\,cm^{-1}$
Pyrocatechol		*o*-Benzoquinone	390	1417
Protocatechuic acid		1-carboxy, 3,4-benzoquinone	390	1300
Dopamine		5,6-indolequinone	465	2455
Dopa		Dopachrome	480	3388
Hydroquinone		Quinhydrone	440	890

Table 2. Activity of the periostracal phenoloxidase in the supernatant and the pellet (3 4 dihydroxyphenylethylamine used as substrate).

	Activity of periostracal phenoloxidase Δ absorbance mg protein^{-1} min^{-1}
Supernatant of homogenate in 0·2 M Tris HCl, buffer pH 7·4	$0{\cdot}012 \pm 0{\cdot}0013$
Pellet treated with 0·5% (w/v) SDS and the resulting supernatant used as enzyme source	$0{\cdot}053 \pm 0{\cdot}0024$
The above supernatant treated with 25 μg trypsin/mg protein	$0{\cdot}075 \pm 0{\cdot}0021$

Table 3. Activity of phenoloxidase from the periostracum of *P. viridis* on various substrates.

Substrates	μmol of substrate oxidized/mg protein/min	Relative velocity
Pyrocatechol	36·00	2·50
Dopamine	30·50	2·10
Dopa	14·50	1·00
Protocatechuic acid	8·46	0·59
Hydroquinone	5·60	0·39
Tyrosine	0·00	0·00
Tyramine	0·00	0·00

3–4 dihydroxyphenylethylamine. The enzyme does not react with monophenols like tyramine and tyrosine.

The hydrolysate of the mantle contains the following phenolic compounds, identified by their R_f values and the colour formed with the spraying reagents: dopa (R_f 0·27), dopamine (R_f 0·59) and a diphenol (R_f 0·89).

4. Discussion

In insects, the enzyme phenoloxidase has been reported to occur both in soluble and insoluble forms. In cases where the enzyme is bound to the membrane and is insoluble, it has been solubilized by the use of detergents (Hackman and Goldberg 1967; Hughes and Price 1974) or by digestion with proteolytic enzymes (Yamazaki 1969; Andersen 1978). In the periostracum of *P. viridis*, though some phenoloxidase activity is released in the supernatant, the enzyme is completely solubilized by the addition of SDS thus indicating that it may be bound to membrane. This enzyme is further activated by trypsin resembling the periostracal phenoloxidase from *Modiolus* (Waite and Wilbur 1976). In contrast, completely soluble phenoloxidases have been reported from the byssal gland of *P. viridis* (Bharathi 1982) and from the ink of cephalopods (Prota *et al* 1981).

It is seen that the periostracal phenoloxidase from *P. viridis* catalyses *o*-diphenolic substrates like pyrocatechol, dopamine, dopa and protocatechuic acid as well as *p*-phenol hydroquinone, thus exhibiting a wide substrate specificity. Due to its catalytic activity with different substrates, the enzyme could exist in a multiple form resembling the phenoloxidase from the byssal gland of *P. viridis* (Bharathi 1982; Bharathi and Ramalingam 1983). Multiple forms of phenoloxidase have also been reported from insects (Hughes and Price 1975; Pau and Kelly 1975). As the enzyme does not oxidize monophenols, there seems to be no monophenolase activity. Similarly the phenoloxidase from *Modiolus* and *Biomphalaria glabrata* shows no activity with monophenols (Waite and Wilbur 1976; Aragao and Bacila 1976). It is of interest to note that while the periostracal phenoloxidase from *P. viridis* shows a high activity with pyrocatechol, the byssal phenoloxidase shows maximum activity with *p*-phenylenediamine and hydroquinone respectively. Such a diversity is also reported in the insect *Drosophila virilis* in which the cuticular and haemolymphal phenoloxidases differ in their substrate preference (Ohnishi 1954; Yamazaki 1969). Thus the present study elucidates the fact that in *P. viridis* the periostracal phenoloxidase differs in its nature and properties from the byssal phenoloxidase. The *o*-quinones involved in the phenolic tanning of the periostracum of molluscs may be derived from the oxidation of tyrosine or dopa (Bubel 1980).

Histochemical studies have revealed the presence of tyrosine and dopa in the periostracum-secreting cells of the mantle (Hillman 1961; Bubel 1976). Similarly, histochemical localization of the phenols from the byssal gland of *Mytilus edulis* revealed the precursor of the tanning phenol to be dopa, which may get degraded to dopamine and catechol before being oxidized to quinone (Ravindranath and Ramalingam 1972). In the present study the occurrence of dopa and dopamine in the mantle of *P. viridis* suggests a similar pathway. Catechol may be the tanning phenol in the mantle for the enzyme shows higher activity to catechol in *in vitro* studies. Thus, the existence of the periostracal phenoloxidase in multiple form and its relation to tanning phenols deserve to be studied further.

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Mitochondrial adenosine triphosphatase of *Penetrocephalus ganapatii* (Cestoda: Pseudophyllidea) in relation to activators and inhibitors

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Abstract. The mitochondrial adenosine triphosphatase of the cestode parasite, *Penetrocephalus ganapatii* exists as a latent form and is activated by dinitrophenol or $MgCl_2$. Irrespective of $MgCl_2$ or dinitrophenol concentrations, the enzyme shows optimum activity at pH 8. Effects of inhibitors on Mg^{2+}, dinitrophenol and Mg^{2+} + dinitrophenol activated ATPase reveal that ATPase is not highly sensitive towards oligomycin and the alteration of the redox state of the respiratory chain components by rotenone, antimycin A, azide and cyanide has little effect on ATPase activity. –SH groups of the enzyme seem to play a limited role in the hydrolysis of ATP, as the enzyme is only partially sensitive to *p*-chloromercuribenzoic acid. Generally, cations inhibit Mg^{2+} stimulated ATPase and activate dinitrophenol stimulated ATPase but most of the anions are inhibitory to dinitrophenol or Mg^{2+} stimulated ATPase.

Keywords. Mitochondria; ATPase; activators; inhibitors.

1. Introduction

The terminal step of oxidative phosphorylation in the respiratory chain is catalyzed by a complex enzyme system called mitochondrial ATPase (Panet and Sanadi 1976; Tzagaloff 1976). This ATPase complex is oligomycin sensitive and activation of ATPase by Mg^{2+} and the uncoupler 2,4-dinitrophenol (DNP) is an universal feature (Chefurka 1981a). The site for synthesis and hydrolysis of ATP is the soluble F_1 unit of this ATPase complex (Penefsky *et al* 1960; Pullman *et al* 1960; Racker 1970). The F_1 ATPase isolated and characterized from a variety of sources displays remarkable similarities in structure and properties (Panet and Sanadi 1976). The characteristics of uncoupler activated ATPase have been thoroughly studied in mammalian liver mitochondria with respect to inhibitors (Lardy *et al* 1958; Bruni *et al* 1965; Lardy and Lin 1969; Beechey *et al* 1967), dependence on cations (Amons *et al* 1968; Cereijo-Santalo 1972) and anions (Veldsema-currie and Slater 1968) and involvement of respiratory chain components (Weiner and Lardy 1974). In parasitic helminths, although a few attempts have been made to study the property of this enzyme in the nematodes *Ascaris lumbricoides* (Hayashi 1973) and *A. suum* (Van den Bossche 1972, 1974) and the cestode *Schistocephalus solidus* (Walker and Barrett 1983), no information is available in other parasitic helminths regarding the properties of this enzyme, particularly in relation to activators and inhibitors. In view of the importance of ATP synthesis and other ATP-dependent functions in these highly specialized anaerobic animals, this paper deals with the effect of activators and inhibitors on the mitochondrial ATPase of a pseudophyllid cestode parasite, *Penetrocephalus ganapatii*.

2. Materials and methods

2.1 *Preparation of mitochondria*

Live parasites of *P. ganapatii* were collected from the intestine of the host fish *Saurida tumbil*. They were washed several times in 0·85% saline before homogenization. A 10% homogenate was prepared in 0·25 M sucrose and centrifuged at 1,000 *g* for 10 min to sediment the cell debris. The supernatant fraction was recentrifuged at 10,000 *g* for 15 min. The sediment obtained was resuspended in the sucrose solution to the original volume and centrifuged again at 10,000 *g* for 15 min. The final pellet containing mitochondria was suspended in a known volume of sucrose solution and used for the enzyme assay. The whole procedure was carried out at 4°C. The presence of mitochondria in the pellet was assessed by assaying the marker enzymes succinate dehydrogenase and cytochrome oxidase.

2.2 *Enzyme assay*

Mitochondrial ATPase (EC 3·6·1·4) activity was measured following the method of Veldsema-currie and Slater (1968). The reaction mixture contained 50 mM Tris-HCl buffer pH 8, 75 mM KCl, 1 mM EDTA, 2·5 mM ATP, mitochondrial suspension and water to a total volume of 1 ml. The reaction was initiated by the addition of 0·1 ml of mitochondrial suspension (150–200 μg protein). The reaction mixtures incubated, after the addition of mitochondrial protein, for periods from 5–30 min showed that ATPase activity was linear up to 15 min. Therefore the reaction mixtures were incubated for 15 min in all cases and then terminated by the addition of 10% (w/v) ice-cold trichloroacetic acid (TCA). The mitochondrial suspension added after the addition of TCA to the reaction mixture constituted the control. After termination of reaction, the precipitate formed was removed by centrifugation at 1,000 *g* for 5 min. The resultant supernatant was analyzed to determine the amount of phosphate liberated. Quantitative estimation of phosphate was carried out following the method of Fiske and Subbarow (1925). Protein was determined according to Lowry *et al* (1951). The specific activity of the enzyme is expressed as nmol P_i liberated/min/mg protein.

2.3 *Chemicals*

ATP, oligomycin, rotenone, *p*-chloromercuribenzoic acid (*p*-CMB), malate, fumarate and 2-oxoglutarate were obtained from Sigma Chemical Co., St. Louis, Missouri, USA. Antimycin A was obtained from Cal Biochem, La Jolla, USA. Malonate was obtained from British Drug House, Poole, England.

3. Results

Freshly prepared mitochondria of *P. ganapatii* showed only low activity of ATPase from pH 7–10·5 with a maximum of 39 ± 3 nmol at pH 8 (figure 1). Various concentrations of $MgCl_2$ at different pH exhibited an activation of ATPase activity but it was found to be concentration and pH dependent (figure 1). The

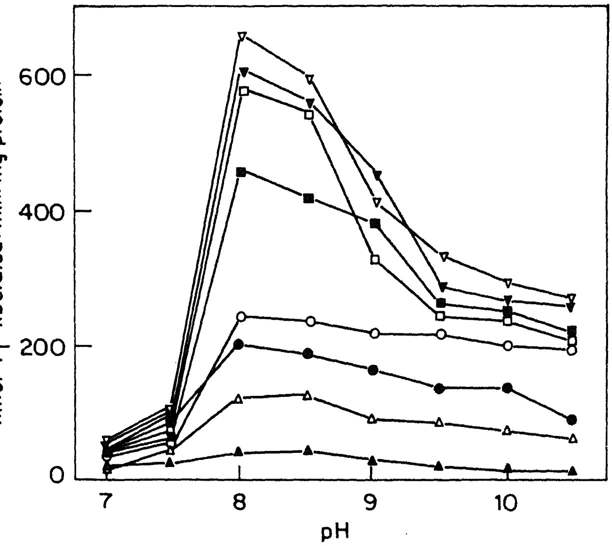

Figure 1. Effects of pH and $MgCl_2$ on the mitochondrial ATPase of *P. ganapatii*. Activities were measured in the reaction mixture described under materials and methods but varying the pH of the buffer and by adding different concentrations of $MgCl_2$.
(▲), No $MgCl_2$; (●), 1 mM $MgCl_2$; (○), 2 mM $MgCl_2$; (■), 3 mM $MgCl_2$; (□), 4 mM $MgCl_2$; (▼), 5 mM $MgCl_2$; (▽), 10 mM $MgCl_2$; (△), 100 mM $MgCl_2$.

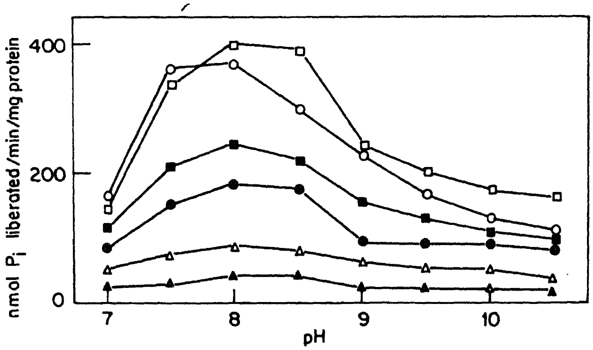

Figure 2. Effects of pH and $MgCl_2$ on the mitochondrial ATPase of *P. ganapatii*. Activities were measured in the reaction mixture described under materials and methods but varying the pH of the buffer and by adding different concentrations of DNP.
(▲), No DNP; (△), 1 μM DNP; (●), 10 μM DNP; (○), 100 μM DNP; (□), 1 mM DNP; (■), 10 mM DNP.

maximum extent of activation was noticed at 10 mM $MgCl_2$ which showed an activity of 660 ± 12 nmol at pH 8. A high concentration of 100 mM $MgCl_2$, however, exhibited a reduction in ATPase activity, which was well below of that obtained for 1 mM $MgCl_2$. Nevertheless, the activity curves showed an optimum pH of 8 irrespective of $MgCl_2$ concentrations.

The data in figure 2 represent the effect of the uncoupler DNP. Different concentrations of DNP were also effective in activating the enzyme but dependent upon

concentration of DNP and pH similar to that of $MgCl_2$. One mM DNP was found to exert maximum activation of ATPase with an activity of 399 ± 7 nmol at pH 8. However, as has been noticed for higher concentrations of $MgCl_2$, a higher concentration of 10 mM DNP showed a diminished ATPase activity.

Different concentrations of $MgCl_2$ and DNP in combinations were also tried at pH 8 of the reaction mixture to find out the optimal $MgCl_2$ and DNP concentrations required to elicit the maximal ATPase activity. The results (figure 3) indicated that the activity was enhanced by lower concentrations of $MgCl_2$ and DNP but diminished at higher concentrations. A maximum ATPase activity of 860 ± 14 nmol was obtained when 5 mM $MgCl_2$ and 100 μM DNP were present in the reaction mixture.

The effects of different inhibitors on the ATPase activity of *P. ganapatii* are shown in table 1. The energy transfer inhibitor oligomycin at concentrations of 1, 5 and 10 μg in the reaction mixture brought out significant but not effective inhibition of Mg^{2+} as well as DNP activated ATPase. However, the effect of oligomycin on the ATPase induced by both Mg^{2+} and DNP was found to be very meagre even at the highest concentration of 10 μg oligomycin. Similarly, the inhibitors of the respiratory chain viz. rotenone (site I), antimycin A (site II), sodium azide (site III) and potassium cyanide (site III) at different concentrations were also found to exert a weak inhibitory effect on the ATPase induced by either Mg^{2+} or DNP or by both. However, the inhibition obtained at 1×10^{-5} M of rotenone and 1×10^{-3} M of cyanide was observed to be substantial when compared to other inhibitors of the respiratory chain. Besides these, the inhibitor *p*-CMB, which binds with the $-SH$ group of the enzyme, had also exhibited a low inhibitory effect on the enzyme induced by Mg^{2+} as well as DNP.

Table 2 shows the effects of anions on the ATPase activity of *P. ganapatii*. With an exception of succinate, which showed a small but significant activation (17%) of the DNP activated ATPase, all other anions had either no effect or inhibitory effects. The ATPase induced by Mg^{2+} ions was strongly inhibited by 2-oxoglutarate. But the effect was seemed to be less on the DNP activated and Mg^{2+} + DNP acti-

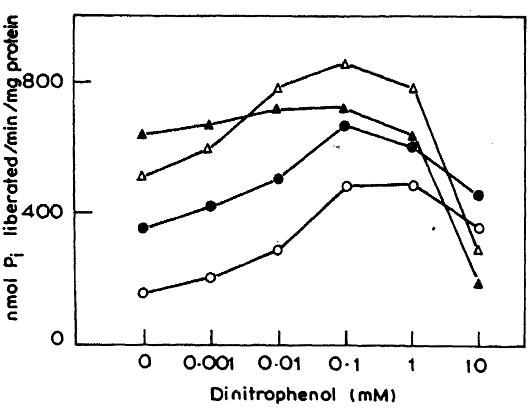

Figure 3. Effects of DNP on the $MgCl_2$ activated mitochondrial ATPase of *P. ganapatii*. Activities were measured in the reaction mixture described under materials and methods. The pH of the assay mixture was 8.
(○), 1 mM $MgCl_2$; (●), 2·5 mM $MgCl_2$; (△), 5 mM $MgCl_2$; (▲), 10 mM $MgCl_2$.

Table 1. Effect of inhibitors on Mg^{2+} and DNP activated mitochondrial ATPase of *P. ganapatii*.

Inhibitors[a]	$MgCl_2$ (10 mM) ATPase[b] activity	Inhibition (%)	DNP (1 mM) ATPase[b] activity	Inhibition (%)	$MgCl_2$+DNP (5 mM) (100 μM) ATPase[b] activity	Inhibition (%)
Control	660 ± 12	0	399 ± 07	0	860 ± 14	0
Oligomycin[c]						
1 μg	577 ± 15	13	297 ± 21	26	867 ± 26[d]	0
5 μg	458 ± 14	31	244 ± 17	39	825 ± 12[d]	4
10 μg	389 ± 26	41	186 ± 39	53	725 ± 19	16
Rotenone[c]						
1×10^{-7} M	608 ± 13	8	362 ± 09	9	854 ± 16[d]	0
1×10^{-6} M	556 ± 26	16	274 ± 23	31	862 ± 20[d]	0
1×10^{-5} M	465 ± 19	30	215 ± 25	46	636 ± 13	26
Antimycin A[c]						
1×10^{-6} M	587 ± 13	11	397 ± 06[d]	0	856 ± 16[d]	0
1×10^{-5} M	503 ± 27	24	377 ± 11	6	846 ± 19[d]	2
1×10^{-4} M	457 ± 17	31	332 ± 21	17	826 ± 22[d]	4
Azide						
1×10^{-4} M	548 ± 27	17	396 ± 12[d]	0	869 ± 18[d]	0
1×10^{-3} M	399 ± 25	40	379 ± 11	5	800 ± 11	17
Cyanide						
1×10^{-4} M	658 ± 12[d]	0	370 ± 10	7	856 ± 09[d]	0
1×10^{-3} M	365 ± 15	45	190 ± 26	52	578 ± 21	33
p-CMB						
1×10^{-4} M	590 ± 09	11	374 ± 16	6	856 ± 18[d]	0
1×10^{-3} M	523 ± 25	21	318 ± 21	20	847 ± 19[d]	2

[a]Inhibitors were added in the reaction mixture at concentrations given above and incubated for 15 min.
[b]The ATPase activity was assayed in the reaction mixture described under materials and methods. The activities are expressed as nmol P_i liberated/min/mg protein. Values are given as Mean ± SD for 5 determinations.
[c]Oligomycin, rotenone and antimycin A were dissolved in ethanol and added in 10 to 50 μl to achieve the desired concentrations. Control tubes received appropriate amount of ethanol alone.
[d]Values are not significantly different from control at 5% level in Student-Newman-Keuls test (Sokal and Rohlf 1969).

vated ATPase. The other anions fumarate and oxalate also showed some inhibitory effect on the ATPase activity of *P. ganapatii*.

Different divalent cations at 10 mM concentration were also used to study their effects. The results revealed that the effects were variable (table 3). When the reaction mixture contained Mg^{2+} ions, the effects of cations were mostly inhibitory falling in the order of $Zn^{2+} > Mn^{2+} > Cd^{2+} > Ni^{2+} > Ba^{2+}$. In contrast to this, when the reaction mixture contained DNP, the enzyme was found to be enhanced by many of the cations, which fall in the order of $Ba^{2+} = Mn^{2+} < Ca^{2+}$. However, there seemed to be no further activation of the enzyme if the reaction

Table 2. Effect of anions on Mg^{2+} and DNP activated mitochondrial ATPase of *P. ganapatii.*

Anions[a] (10 mM)	MgCl$_2$ (10 mM) ATPase[b] activity	Activity (%)	DNP (1 mM) ATPase[b] activity	Activity (%)	MgCl$_2$ + DNP (5 mM) (100 μM) ATPase[b] activity	Activity (%)
Control	688 ± 14	100	402 ± 09	100	842 ± 08	100
L-Glutamate	658 ± 37[c]	95	401 ± 12[c]	100	830 ± 14[c]	99
Acetate	661 ± 24[c]	96	403 ± 14[c]	100	844 ± 13[c]	100
Oxalate	495 ± 25	72	274 ± 32	68	695 ± 19	83
Succinate	664 ± 09[c]	96	471 ± 40	117	839 ± 16[c]	100
L-Malate	598 ± 15	87	396 ± 34[c]	99	856 ± 22[c]	102
Fumarate	456 ± 21	66	238 ± 36	59	556 ± 11	66
Malonate	562 ± 26	82	382 ± 15	95	837 ± 14[c]	100
2-Oxoglutarate	048 ± 11	7	164 ± 16	40	415 ± 21	49

[a]Anions were added in the reaction mixture and incubated for 15 min.
[b]The ATPase activity was assayed in the reaction mixture described under materials and methods. The activities are expressed as nmol P_i liberated/min/mg protein. Values are given as Mean ± SD for 5 determinations.
[c]Values are not significantly different from control at 5% level in Student-Newman-Keuls test (Sokal and Rohlf 1969).

Table 3. Effect of cations on Mg^{2+} and DNP activated mitochondrial ATPase of *P. ganapatii.*

Cations[a] (10 mM)	MgCl$_2$ (10 mM) ATPase[b] activity	Activity (%)	DNP (1 mM) ATPase[b] activity	Activity (%)	MgCl$_2$ + DNP (5 mM) (100 μM) ATPase[b] activity	Activity (%)
Control	645 ± 01	100	398 ± 11	100	851 ± 14	100
Mn^{2+}	396 ± 19	61	542 ± 31	136	731 ± 12	86
Ca^{2+}	647 ± 15[c]	100	707 ± 33	177	766 ± 16	90
Ba^{2+}	498 ± 45	77	540 ± 27	135	842 ± 21[c]	99
Cd^{2+}	429 ± 26	67	401 ± 09[c]	100	664 ± 19	78
Ni^{2+}	481 ± 15	75	403 ± 10[c]	101	846 ± 13[c]	100
Zn^{2+}	292 ± 30	45	342 ± 29	86	544 ± 24	64

[a]Cations were added in the reaction mixture and incubated for 15 min.
[b]The ATPase activity was assayed in the reaction mixture described under materials and methods. The activities are expressed as nmol P_i liberated/min/mg protein. Values are given as Mean ± SD for 5 determinations.
[c]Values are not significantly different from control at 5% level in Student-Newman-Keuls test (Sokal and Rohlf 1969).

mixture contained both Mg^{2+} and DNP, although there was some inhibitory effect by Zn^{2+} and Cd^{2+} ions.

4. Discussion

The data presented show that mitochondrial ATPase of *P. ganapatii* exists in a masked state and is unmasked by the addition of Mg^{2+} or DNP in the reaction

mixture. This property of the enzyme is in line with the observations made in a variety of mammalian and bacterial sources (Panet and Sanadi 1976). Possibly the masked state of the enzyme is due to the presence of an endogenous trypsin sensitive protein inhibitor in the ATPase complex as has been noticed for the beef heart mitochondria (Pullman and Monroy 1963).

In the present study, the Mg^{2+} activated ATPase is stimulated further by DNP, atleast in the lower concentrations (figure 3). Similar results have also been reported for the ATPase of housefly mitochondria (Chefurka 1981c) and turtle heart mitochondria (Rotermund and Previtera 1970). Probably this may be due to the different mechanisms of activation of the enzyme by these chemicals. It has been reported that Mg^{2+} acts as a chelator in the enzyme substrate interaction to enhance the activity (Lardy and Wellman 1953; Myers and Slater 1957a; Selwyn 1968), whereas the DNP is reported to activate the enzyme by uncoupling the coupled mitochondria (Lardy and Elvehjem 1945; Cooper and Lehninger 1957; Hall and Palmer 1969).

It is rather surprising to note that the mitochondria of *P. ganapatii* show relatively higher activity in the presence of Mg^{2+} than DNP. In contrast, DNP is found to be more effective in activating the enzyme of mammalian mitochondria (Myers and Slater 1957a). However, this does not seem to be peculiar to this parasite alone. Earlier studies on helminth parasites have also reported such discrepancies with regard to Mg^{2+} as well as DNP activation of the enzyme. While the mitochondrial ATPase of adult *A. lumbricoides* shows activation only by Mg^{2+} and not by DNP (Hayashi 1973), the ATPase of adult *Taenia taeniaeformis* shows no activation either by Mg^{2+} or by DNP, although activation of the enzyme by these chemicals could be noticed from the mitochondria of the larval stage (Weinbach and Von Brand 1970). It is difficult to speculate the possible reason for this discrepancy, as there are no apparent differences in the nature of the assay mixture and mitochondrial isolation medium used in these studies. However, this may indicate the differences in the permeability of the mitochondrial membrane of these helminths.

The mitochondria of *P. ganapatii* also show an unique property of having only one pH optimum of 8 for both Mg^{2+} and DNP activated ATPase. More than one optimum pH in alkaline range has been reported for the rat liver mitochondrial ATPase (Myers and Slater 1957a) and there have been distinct pH optima for Mg^{2+} (8·5) and DNP (7·4) activated ATPase of the heart mitochondria of the turtle *Chrysemys picta* (Rotermund and Previtera 1970). The ATPase of *P. ganapatii* is also different from the housefly which exhibits an optimum pH of 6·5 in the acidic range (Chefurka 1981b).

The energy transfer inhibitor oligomycin was first introduced by Lardy *et al* (1958) as an inhibitor of oxidative phosphorylation. Subsequent experiments by Weiner and Lardy (1974) have shown that 1 μM of oligomycin is sufficient to bring out the complete inhibition of ATPase activity. In the present study, it has been noticed that the inhibition is very weak even at a higher concentration of 10 μM oligomycin, suggesting that only a portion of the ATPase of *P. ganapatii* is sensitive towards oligomycin.

The present investigation also shows that the respiratory inhibitors are not effective in inhibiting the ATPase of *P. ganapatii*. Rotenone, antimycin A, azide and cyanide at concentrations that are highly inhibitory to the respiratory chain have produced only a weak or moderate inhibitory effect on the ATPase of *P. ganapatii*. Weiner and Lardy (1974) and Chefurka (1981a) have noticed that inhibition of the

ATPase by respiratory inhibitors is dependent upon the metabolic state of the mitochondria and the nature of the uncoupler used. In view of this, it may be assumed that, under the conditions of our experiments, the alteration of the redox state of the mitochondria by respiratory inhibitors has little effect on the ATPase of *P. ganapatii*. Further, *p*-CMB, the well known thiol group inhibitor, was also found to be a weak inhibitor of the ATPase of *P. ganapatii*. This indicates that −SH group may not play a major role in the hydrolysis of ATP. This observation is in contrast to liver mitochondria, where *p*-CMB is reported to have strong inhibitory effect on ATPase (Myers and Slater 1957b).

The ATPase of *P. ganapatii* induced by Mg^{2+} or DNP or by both is inhibited by some of the anions. Inhibition by anions has also been noticed in DNP activated ATPase of rat liver mitochondria (Veldsema-currie and Slater 1968). The inhibition may be because of the competition by these anions with Mg^{2+} or DNP for penetration into the mitochondria. However, the interesting observation with anions is the activation of DNP-activated ATPase by succinate. Although the reason for this activation is not known at present, this property of *P. ganapatii* mitochondrial ATPase is similar to that reported for fly muscle mitochondrial ATPase (Chefurka 1981c).

It is interesting to note that Mg^{2+} activated and DNP activated ATPase vary in their response towards the cations. While the former is inhibited by most of the cations the latter showed activation by some of the cations. However, only inhibitory effect by some of the cations was noticed when both Mg^{2+} and DNP were present in the reaction mixture. The activation of DNP-activated ATPase by metal ions may be attributed to the chelating effect of these ions in the enzyme substrate interaction similar to that of Mg^{2+} ions (loc. cit). However, the inhibitory effect of the divalent cations on the Mg^{2+} stimulated ATPase could be due to the binding of the cations with the enzyme surface (Ulrich 1964).

It would appear, from the foregoing account, that the properties of mitochondrial ATPase of *P. ganapatii* differ not only from mammalian and insect mitochondria but also from parasites, though it resembles them to certain extent.

Acknowledgement

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Campoletis chlorideae Uchida (Hymenoptera: Ichneumonidae) as a parasite of *Helicoverpa armigera* (Hub.) (Lepidoptera: Noctuidae) in southwest India

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Abstract. *Campoletis chlorideae* Uchida was the most common parasite emerging from the larvae of *Helicoverpa armigera* (Hub.) collected during 1974–83 from almost all of its crop and weed hosts from Andhra Pradesh, Karnataka and Maharashtra. In most years, parasitism was highest in September and lowest in May. At ICRISAT Center, during 1977–83, the average percentage parasitism of first to third instar larvae, which are only parasitised by *Campoletis chlorideae*, was 44·2 on sorghum, 33·1 on chickpea, 32·6 on pearl millet, 7·1 on groundnut and 4·2 on pigeonpea. Parasitism in pesticide-treated crops was lower than in untreated crops. Ten species of hyperparasites were recorded from cocoons of *Campoletis chlorideae*. Hyperparasitism was around 40% on cereals and 10% on legumes.

Keywords. Parasitism; hyperparasitism; *Campoletis chlorideae*; *Helicoverpa armigera*.

1. Introduction

Campoletis chlorideae Uchida, misidentified until 1980 successively as *Horogenes fenestralis* Holmgren (Tikar and Thakare 1961), *Campoletis perdistinctus* Viereck (Gangrade 1964), *Ecphoropsis perdistinctus* Viereck (Mathur and Dharmadhikari 1970) and *Diadegma* sp. (Bhatnagar and Davies 1979), is one of the most common larval parasites of *Helicoverpa armigera* (Hub.) in India (Bilapate *et al* 1979; Yadav *et al* 1982). The parasite deposits eggs singly in first or second instar host larvae, which usually die in the third or fourth instar. The fully grown parasite larva leaves the host to spin a cocoon and pupate on the plant. A detailed biology and ecology of this parasite in India is described by Patel and Patel (1972) and of the related species, *C. sonorensis* (Cameron), in the USA by Danks *et al* (1979). This paper describes the field observations on the parasite and its role in the natural control of *H. armigera*.

2. Materials and methods

During 1974–83, *H. armigera* larvae were collected from a range of crops and weeds from large areas of the states of Andhra Pradesh, Karnataka and Maharashtra (figure 1). They were reared in the laboratory at 25 ± 2°C and 60–70% RH to record parasite emergence.

H. armigera larvae were also collected at ICRISAT Center, Patancheru from sorghum, pearl millet, groundnut, pigeonpea and chickpea. Unlike in the survey, they were grouped as 1–3 instar and 4–6 instar larvae to record parasitism.

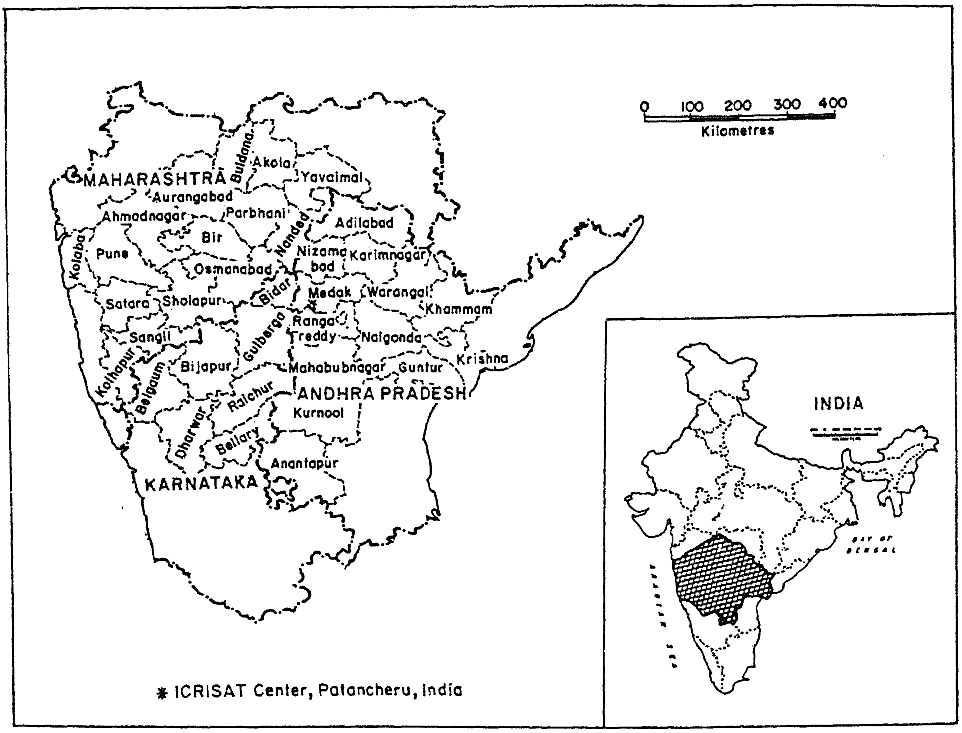

Figure 1. Area covered in survey for *Helicoverpa* parasite in India (1974–85).

Pesticide treated pigeonpea and chickpea were sampled separately. Cocoons of *C. chlorideae* were collected to investigate the extent of hyperparasitism.

The total per cent and the degree of parasitism by *C. chlorideae* alone in *H. armigera* larvae were estimated irrespective of the larval instar for the surveyed area. However, for ICRISAT Center the parasitism by *C. chlorideae* in 1–3 instar larvae was estimated separately to indicate precisely its impact on the mortality of *H. armigera* on ICRISAT crops.

3. Results

3.1 *C. chlorideae on different crops and weeds*

C. chlorideae parasitised *H. armigera* larvae on almost all the crops and weeds covered in the survey at all times of the year (table 1). It was, however, most abundant in cereal crops where it parasitised 23·0, 16·6 and 4·1% of *H. armigera* larvae on sorghum, pearl millet, and maize respectively. On these crops *C. chlorideae* accounted for 60–85% of the total larval parasitism of *H. armigera.*

Among the legumes, the parasitism of *H. armigera* larvae by *C. chlorideae* was highest on chickpea (16·3%), followed by soybean (12·8%), groundnut (4·2%) and cowpea (2·4%). These figures represented 40–80% of the total parasitism on these crops.

Table 1. Average parasitism (%) of larvae of *H. armigera* by *C. chlorideae* in relation to total larval parasitism in different crops and weeds and its distribution across months in Andhra Pradesh, Karnataka and Maharashtra, 1974–85.

	Overall larval parasitism@ (%)	Parasitism by *C. chlorideae* alone (%)	*C. chlorideae* parasitism across months											
			Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May.
Cereals														
Sorghum	27·2 (37003)	23·0	0·0	0·0	16·3	26·6	0·0	9·7	21·1	0·0	3·4	0·0	0·0	0·0
Pearl millet	22·6 (1423)	16·6	—	—	16·5	17·1	—	—	18·7	—	—	—	—	0·0
Maize	6·4 (2241)	4·1	—	0·0	4·6	3·1	—	10·0	—	25·0	0·0	—	0·0	0·0
Legumes														
Pigeonpea	14·5 (89602)	0·9	—	0·2	0·0	2·6	3·4	0·7	0·9	0·7	0·1	0·4	0·0	0·0
Chickpea	20·6 (60793)	16·3	0·0	1·3	6·1	15·1	27·9	17·3	24·1	16·3	14·0	11·3	43·7	1·4
Groundnut	12·8 (7351)	4·2	0·2	1·2	8·5	7·5	—	—	—	9·9	2·9	1·4	4·7	2·1
Cowpea	4·0 (6316)	2·4	—	1·5	0·0	—	—	17·1	—	—	—	0·0	1·6	3·0
Mungbean	4·4 (682)	0·1	—	—	0·0	4·5	—	—	—	—	—	—	—	—
Soybean	26·3 (133)	12·8	—	—	—	0·0	0·0	13·9	—	—	—	—	—	—
Pea	63·6 (11)	63·6	—	—	—	—	—	—	—	63·6	—	—	—	—
Oilseeds/fibre														
Safflower	31·5 (5316)	21·1	—	—	50·0	—	—	14·7	22·9	20·5	82·2	—	—	—
Sunflower	8·3 (96)	5·2	—	—	0·0	8·9	0·0	—	—	0·0	—	—	—	—
Linseed	24·8 (2060)	6·8	—	—	—	—	—	44·4	6·4	6·9	—	—	—	—
Cotton	12·8 (201)	12·8	—	—	—	12·8	—	—	—	—	—	—	—	—
Vegetable														
Tomato	8·5 (2613)	4·4	0·0	0·9	0·7	4·0	—	0·0	—	0·0	0·0	19·0	0·0	2·1
Weeds														
Cardiospermum halicacabum	14·3 (7)	14·3	—	—	—	—	—	—	—	—	14·3	—	—	—
Cleoma gynandra	37·6 (2301)	4·6	0·0	0·2	1·1	—	—	—	—	—	—	—	64·0	6·7
Gomphrena celosioides	30·9 (5785)	14·6	0·0	0·0	10·8	38·5	44·2	—	—	—	0·0	—	—	0·0
Hibiscus panduraeformis	14·3 (28)	3·6	—	—	—	—	—	—	—	—	3·6	—	—	—
Sesbania bispinosa	27·0 (679)	3·7	—	—	—	21·7	1·8	—	—	—	—	—	0·0	—
Cumulative parasitism			0·1 (1146)	0·6 (7294)	11·3 (16114)	25·7 (30136)	12·8 (12012)	4·4 (52493)	8·3 (31384)	9·2 (33860)	10·6 (16613)	8·0 (12612)	6·6 (5740)	2·3 (5237)

@Total parasitism in larvae inclusive of all parasites, insects and nematodes. Numbers in parentheses refer to a total number of larvae collected, 1974–85.

On safflower, tomato and sunflower *C. chlorideae* parasitised 21·1, 4·4 and 5·2% of the *H. armigera* larvae respectively. Substantial parasitism was also recorded on weeds particularly *Gomphrena celosioides* (L.).

3.2 *C. chlorideae on crops at ICRISAT Center*

H. armigera occurs throughout the year at ICRISAT Center (Pawar *et al* 1984) from July–September on groundnut, sorghum and pearl millet and from October–February on pigeonpea and chickpea (figure 2A). Small numbers are found on the post-rainy crops of groundnut and also of sorghum and pearl millet (December–May). These numbers, however, decline since the crops at ICRISAT Center are progressively cleared by May.

C. chlorideae parasitised *H. armigera* on all these crops. The average rate of parasitism varied with the crop type but was highest on sorghum (44·2%); this was followed by chickpea (33·1%), pearl millet (32·6%), groundnut (7·1%) and pigeonpea (4·2%). Parasitism was highest in September, when the overall parasitism in 1–3 instar larvae reached 46%, but it tended to decline from September to

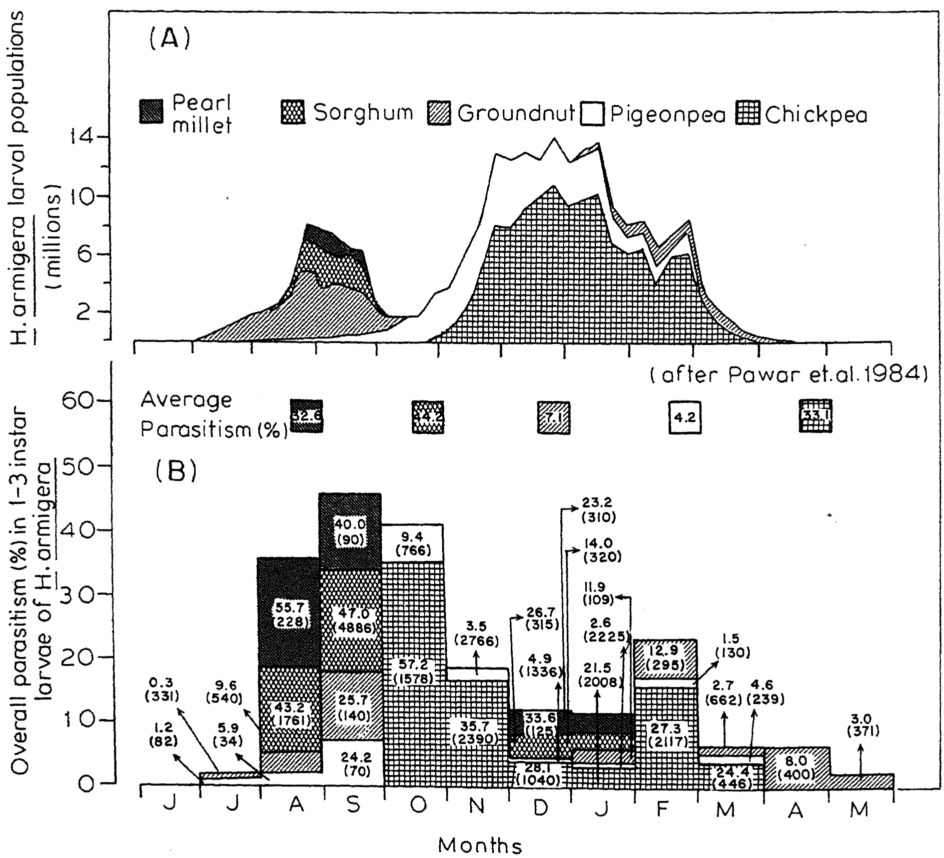

Figure 2. **A.** Average populations of *H. armigera* larvae on crops. **B.** Average parasitism (%) by *C. chlorideae* in 1–3 larvae of *H. armigera*, ICRISAT, 1977–83.

January as the *H. armigera* populations increased. This was followed by a slight rise in the overall parasitism during February as the *H. armigera* populations receded.

3.3 *Effect of insecticide application on parasitism*

The crops of pigeonpea and chickpea at ICRISAT Center receive usually 2–3 applications of endosulfan (0·7 kg AI/ha) to control *H. armigera.* During 1976–79, the average parasitism of small larvae was recorded at 2·8% in treated and 4% in untreated pigeonpeas and 12·1% in treated and 31·2% in untreated chickpeas (table 2).

3.4 *Other hosts of C. chlorideae*

Among the other 28 species of insect larvae that were also collected during the survey, *C. chlorideae* emerged from *H. peltigera* Schiff from safflower (6·2%, n=5192), *Acanthospermum hispidum* DC. (1·6%, n=2072) and *H. assulta* Guenee from *Datura metel* L. (3·2%, n=5147), *Adisura stigmatica* Warr. from pigeonpea (3%, n=668) and *Spodoptera litura* Fab. from groundnut (5%, n=1032).

3.5 *Hyperparasites of C. chlorideae*

Ten species belonging to 5 families of Hymenoptera were recorded emerging from the cocoons of *C. chlorideae.* They were collected from sorghum, pearl millet, groundnut, chickpea and pigeonpea (table 3). Hyperparasitism averaged 39·1% on sorghum, 43·1% on pearl millet, 14·6% on groundnut, 9·8% on chickpea and 5% on pigeonpea. Hyperparasites *Pteromalus* spp. appeared on all these crops, but were most active on the cereals. Hyperparasites were also observed on groundnut and pigeonpea. On chickpea *Brachymeria* spp. followed by a species of *Eurytoma* were the most common hyperparasites.

Table 2. Comparison of *C. chlorideae* parasitism in *H. armigera* larvae in sprayed and unsprayed fields, ICRISAT Center, 1976–79.

	Per cent parasitism in 1–3 instar larvae			
	Pigeonpea		Chickpea	
Year	Sprayed	Unsprayed	Sprayed	Unsprayed
1976–77	2·8	4·0	14·1	26·3
	(1291)	(1001)	(972)	(350)
1977–78	4·4	4·6	11·5	28·4
	(1200)	(900)	(637)	(285)
1978–79	1·8	3·6	10·3	33·3
	(1950)	(1500)	(832)	(1212)
Mean	2·8	4·0	12·1	31·2
	(4441)	(3401)	(2441)	(1847)
SE±(M)	0·76	0·29	1·12	2·07

Numbers in parentheses are a total number of larvae collected.

Table 3. Hyperparasites with their parasitism (%) of the cocoons of *C. chlorideae* on some crops, at ICRISAT Center, 1977–82.

	Hyperparasites	Sorghum	Pearl millet	Groundnut	Pigeon pea	Chick pea
Chalcididae	*Brachymeria* sp. nr. *apantelesi* Risbec *B.* sp. (nr. *persica* Masi) *B. wittei* (Schmitz), and *Brachymeria* sp.	2·6	0·0	0·0	0·0	4·3
Eulophidae	*Nesolynx javanica* (Ferr.)	1·3	0·0	0·0	0·0	0·0
Eurytomidae	*Eurytoma* sp.	1·1	0·0	0·0	0·0	3·8
Ichneumonidae	*Hemiteles* sp.	4·8	0·0	0·0	0·0	0·7
Pteromalidae	*Pteromalus semotus* (Walker) *Pteromalus* sp., and *Eupteromalus parnarae* Gahan	29·3	43·1	14·6	5·0	0·5
Total hyperparasitism		39·1	43·1	14·6	5·0	9·8
Total cocoons collected		(1510)	(51)	(62)	(20)	(2821)

4. Discussion

C. chlorideae is the most common larval parasite among 25 insect parasites that have been recorded on *H. armigera* (Bhatnagar *et al* 1982). It is active on most of the crop and weed hosts of *H. armigera* in Andhra Pradesh, Karnataka and Maharashtra and in other parts of India on potato, cotton and lucerne (Yadhav *et al* 1982). Battu (1977) recorded a *Campoletis* sp. parasitising *S. litura* on cauliflower. *C. chlorideae* is probably capable of parasitising *H. armigera* on all of its hosts of which there are some 157 in India (Bhatnagar and Davies 1978). However, its success may vary between them.

The overall decline in the percentage parasitism by *C. chlorideae* from September to January may be attributed to the relatively higher rate of multiplication of *H. armigera* on pigeonpea and chickpea compared with other hosts during this time (Jayaraj 1981). Furthermore, the survival rate of *C. chlorideae* is lower during cooler months particularly December–January. Patel and Patel (1972) and Nikam and Basarkar (1978) showed that *C. chlorideae* suffers high pupal mortality at temperatures below 27°C and above 31°C.

The Commonwealth Institute of Entomology, London confirmed that *C. chlorideae* on *Heliothis* spp. in India was earlier misidentified as a species of *Diadegma*. P K Nikam (unpublished results) also confirmed this, and reported that the *Diadegma* spp. which have been recorded on *A. stigmatica* and *S. litura* are in fact *C. chlorideae* but not those recorded on *Exelastis atomosa* Walk. and *Perigea serva* Walk. It appears that *C. chlorideae* has a narrow host range and has preference for *H. armigera*. Pawar *et al* (1985) reported that *C. chlorideae* parasitised 22·3% (n = 4147) of *H. armigera* larvae compared with 6·2% (n = 5192) of *H. peltigera* larvae on safflower. This was based on a collection of both small and large larvae. This preference for *H. armigera* would certainly confer a relative advantage if large scale release of this parasite for biological control of *H. armigera* is envisaged.

C. chlorideae suffers considerable pupal mortality from hyperparasites particularly on cereals. On chickpea, the lower hyperparasite activity could be due to the acid

exudate of the plants, which is known to deter many insects from visiting this crop (Bhatnagar *et al* 1982). Hyperparasitism may be an important factor in the success of *C. chlorideae* as a biocontrol agent. A study of the factors affecting the impact of hyperparasites on *C. chlorideae* would be necessary before mass release of artificially reared *C. chlorideae* is attempted to control *H. armigera.*

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Relative development and voracity of six species of Aphidophagous syrphids in cruciferous crops

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Abstract. The development and voracity of 6 species of syrphid are described and contrasted. *Eupeodes* (*Macrosyrphus*) *confrator* was larger than the other species as an adult and as a larva, and had a greater daily voracity. Size contributed greatly towards variation in longevity and voracity. All species were similar in the time-course of feeding behaviour.

Keywords. Aphidophagous syrphids; cruciferous crops; development; voracity.

1. Introduction

In the Gangetic plain and the higher altitude parts of India, *Lipaphis erysimi* (Kalt.) is a well-known aphid pest of cruciferous crops such as mustard, cabbage, cauliflower and radish (Bakhetia 1983; Ghosh and Mitra 1983). Winter crops of these vegetables and oilseeds (mustard) are seldom spared from attack by this aphid.

Although as many as 14 species of Syrphidae have been recorded as feeding on *L. erysimi* in cruciferous crops, the commonest are typically *Betasyrphus serarius* Wied., *Episyrphus balteatus* DeGeer, *Ischiodon scutellaris* Fabr., and *Eupeodes* (*Macrosyrphus*) *confrator* Wied. Various authors have studied usually single species, recording their voracity and development (Bhatia and Shaffi 1932; Rahman 1940; Rao 1969; Roy and Bose 1977; Agarwala *et al* 1981), but no experiments have compared species to gain a more comprehensive understanding of the role of syrphid larvae in aphid predation. The experiments reported here were designed to assess the predatory potential of 6 syrphid species (the above 4, plus *Allograpta javana* Wied. and *Episyrphus alternans* Macquart) feeding on *L. erysimi*.

2. Materials and methods

Individual eggs of each of the 6 syrphid species were collected from aphid-infested crops of mustard, cabbage, cauliflower or radish. Each of the eggs was placed on a filter paper in a clean dry Petri dish. A wad of moistened cotton was provided and replaced each day to maintain humidity. A known number of aphids of known instars (normally 3rd or 4th) and weights was provided each day, and observations were taken on developmental and voracity by noting the duration of each developmental stage (egg, larva, puparium) and by making daily measurements of the weight and number of aphids eaten. The lengths of larva and adult were recorded. Larval length was recorded twice, once upon emergence and again at the quiescent period before pupation. In both cases the larvae were measured when

fully extended using a mm scale. Growth rate was calculated as:

$$(\log_{10} [\text{final length} - \log_{10} \text{initial length}])/\text{days}.$$

The experiment was set up with 6–9 replications in laboratory conditions, when average maximum and minimum temperatures were 24 and 14°C respectively and relative humidities 37 and 67%. There was no mortality of larvae during the experiment.

All the data were analysed using SPSS-X programs. Two of these programs were used: MANOVA, nearly always in a two-way species × sex unbalanced analysis of variance using the method of fitted constants; and ONEWAY, a one-way analysis of variance generally using a priori contrasts.

3. Results

Mean values for measured variables are given in table 1. An initial general point is that there do not appear to be any substantial differences between species in the relative amount of feeding done during each stage of larval development. Figure 1 (A) is a plot of the proportion of feeding done at different stages of larval life, and it can be seen that all species are similar in their curves. Figure 1 (B) plots the same data on semi-probability paper, demonstrating further that the amount of feeding on each day of larval development is predictable and constant between species.

The number of aphids eaten varies according to the instar of aphid offered. Many more small 2nd and 3rd instar aphids are required to complete development than the larger 4th instar aphids (figure 2). The further analysis below considers the weight of aphid mass eaten, rather than the number of aphids, in order to control the aphid size.

The statistical analysis is presented as a series of questions for clarity.

Table 1. Mean values of recorded measurements.

	1	2	3	4	5	6	7	8	9
A. javana	m	11·0	8·2	12·5	136·0	133·5	0·10	0·11	21·5
	f	10·9	8·5	10·0	99·6	101·8	0·11	0·13	19·5
B. serarius	m	13·4	9·2	13·2	126·5	124·0	0·12	0·10	22·5
	f	12·2	8·9	8·3	76·7	83·6	0·12	0·12	22·2
E. alternans	m	11·7	8·8	10·4	75·6	79·4	0·15	0·15	17·0
	f	11·1	8·7	8·4	69·9	78·6	0·13	0·14	19·6
E. balteatus	m	10·9	8·4	9·9	88·3	92·1	0·12	0·13	19·5
	f	10·8	8·7	8·2	79·1	82·9	0·11	0·13	21·3
I. scutellaris	m	11·9	8·5	9·8	72·5	74·6	0·15	0·15	19·2
	f	11·4	8·1	11·1	97·6	98·9	0·12	0·13	20·2
E. confrator	m	15·0	11·3	19·2	172·7	151·4	0·12	0·09	22·0
	f	14·4	11·3	18·1	180·5	162·9	0·11	0·08	22·8

1, Sex (m = male, f = female); 2, final size of third instar larva (mm); 3, length of adult (mm); 4, average daily voracity (weight of aphids per day in mg); 5, average total voracity (biomass of aphids eaten, in mg); 6, total voracity adjusted for differences in initial larval size (size upon emergence from egg); 7, growth rate (mm per day on log scale); 8, growth rate adjusted for differences in final larval size; 9, development time, egg to adult (in days).

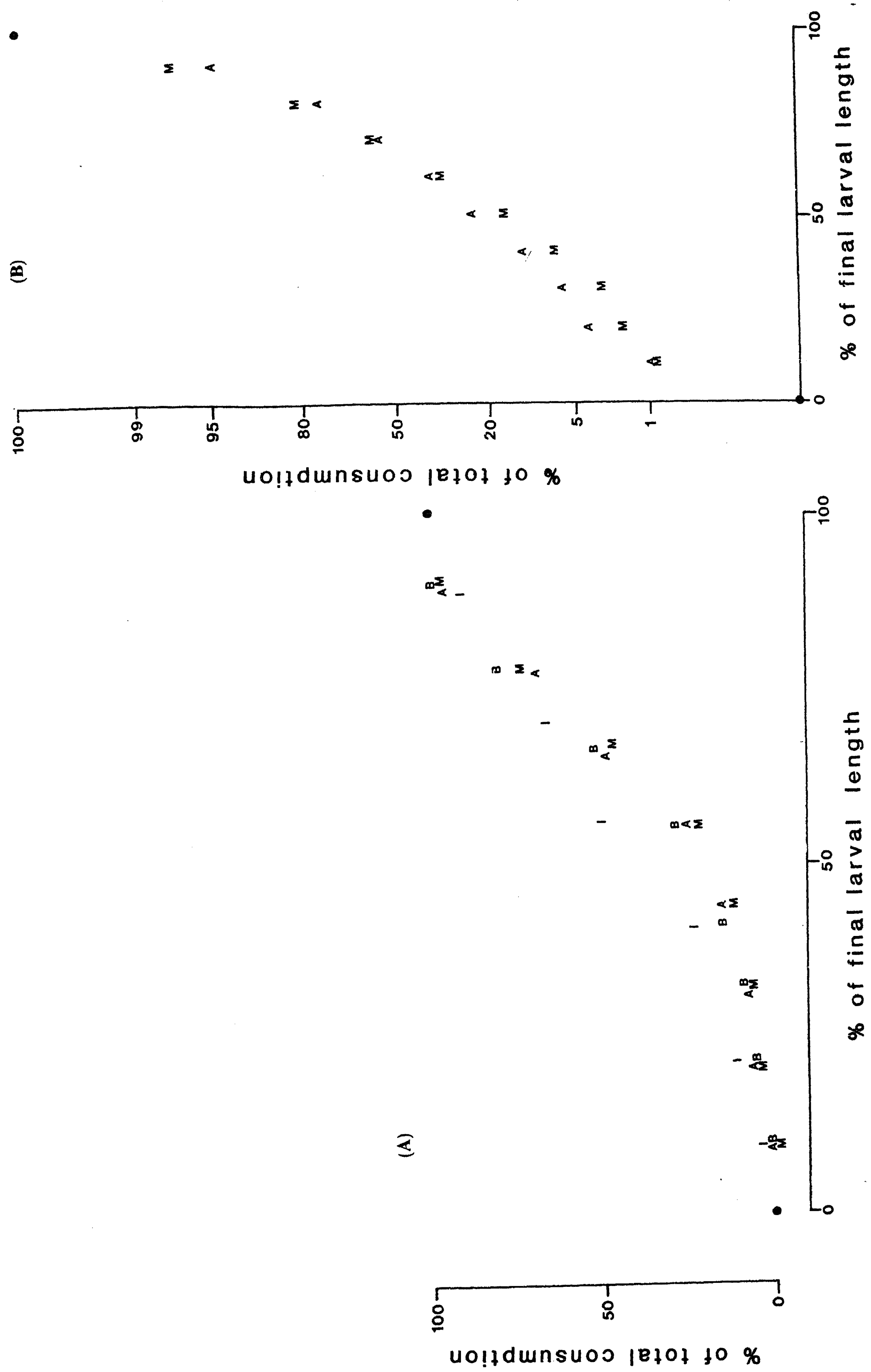

Figure 1. **A.** Development of and predation by larvae of 4 syrphid species. A, *A. javana*; B, *B. serarius*; I, *I. scutellaris*; M, *E. (M.) confrator*. **B.** The same data for two species plotted on probability paper: a slight curve probably is the best fit. A, *A. javana*; M, *E. (M.) confrator*.

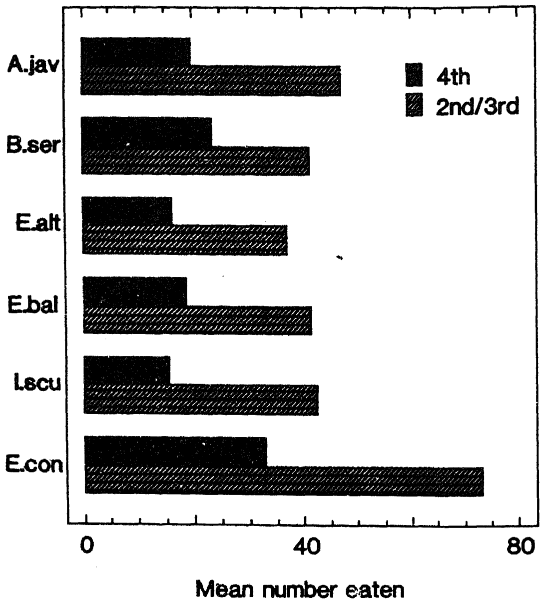

Figure 2. Comparison of the numbers of 4th instar *L. erysimi* as opposed to 2nd + 3rd instar, eaten by larvae of the 6 species of syrphid.

3.1 *Is size of all the species same?*

Adults species are significantly different in size ($F_{5,33} = 27{\cdot}35$, $P < 0{\cdot}001$), but as an overall effect the sexes do not differ in size ($F_{1,33} = 0{\cdot}07$, not significant). The interaction term is not significant ($F_{5,33} = 0{\cdot}38$), implying that the differences between the sexes in adult size are not different between species.

Since *a posteriori* tests are required to establish which species are different, an SNK procedure was used (Sokal and Rohlf 1969). This showed that *E.* (*M.*) *confrator* is significantly larger than all other species.

All the larval stage species are similarly significantly different in size ($F_{5,33} = 13{\cdot}11$, $P < 0{\cdot}001$), but neither sexes ($F_{1,33} = 2{\cdot}16$, not significant) nor the interaction ($F_{5,33} = 0{\cdot}22$, not significant) was significant. Again a posteriori SNK tests showed that *E.* (*M.*) *confrator* is significantly larger than all other species, and that *B. serarius* is larger than *E. alternans*, *E. balteatus* and *A. javana*.

Having found these differences, where appropriate we now set up an a priori contrast of *E.* (*M.*) *confrator* vs the rest, anticipating that size will cause significant differences among species for other measured variables.

3.2 *Do species differ in development time (egg to adult) or growth rate?*

Species differ significantly in development time ($F_{5,33} = 4{\cdot}74$, $P < 0{\cdot}001$), but there are no significant differences between the sexes ($F_{5,33} = 2{\cdot}11$, not significant) and the interaction term is non-significant ($F_{5,33} = 0{\cdot}45$). In a one-way ANOVA for development time, the contrast of *E.* (*M.*) *confrator* vs the rest is not significant.

The same pattern is evident when considering growth rates, where species differ ($F_{5,33} = 2{\cdot}53$, $P < 0{\cdot}05$), but sexes do not ($F_{1,33} = 2{\cdot}17$, not significant) and there is

no interaction ($F_{5,33}=0{\cdot}83$, not significant). A one-way ANOVA with a priori contrast of *E.* (*M.*) *confrator* vs the others shows that all the difference between species resides in this contrast ($P=0{\cdot}023$).

We then asked whether growth-rate differences were related to size differences between larvae, using an analysis of covariance with final larval size as covariate. The covariate has a highly significant regression on growth rate ($F_{1,32}=37{\cdot}7$, $P<0{\cdot}001$), but the residuals still show species differences ($F_{5,32}=6{\cdot}8$, $P<0{\cdot}001$): the effect of sex and the species × sex interaction remain non-significant. Therefore final larval size, while having a significant relationship with growth rate, fails to remove all the differences in growth rate between species.

3.3 *Does voracity differ between species?*

Average daily voracity (weight of aphids eaten per day) differs significantly between species ($F_{5,33}=13{\cdot}06$, $P<0{\cdot}001$), and there is a high variance ratio for the differences between the sexes ($F_{1,33}=3{\cdot}47$, $P=0{\cdot}07$) indicating that male larvae have a higher daily intake than females (table 1). A single classification ANOVA was performed with the contrast *E.* (*M.*) *confrator* vs the rest: this contrast was highly significant ($F_{4,39}=59{\cdot}14$), and no other differences remained ('rest', $F_{4,39}=0{\cdot}53$). Thus, the differences in daily voracity between species are all accounted for by one species, *E.* (*M.*) *confrator*, which has a higher daily intake rate than other species. All other species have the same daily voracity.

To determine whether this difference is entirely due to the size difference between *E.* (*M.*) *confrator* and the other species, an analysis of covariance was performed using final larval size as covariate. The covariate showed only a weak effect on daily voracity ($F_{1,32}=3{\cdot}62$, $P=0{\cdot}066$), species differences remaining ($F_{5,32}=4{\cdot}36$, $P<0{\cdot}01$). Thus the size difference does not account for the differences in daily voracity.

Total voracity over the entire larval life also differ between species ($F_{5,33}=20{\cdot}24$, $P<0{\cdot}001$); the overall difference between sexes was not significant ($F_{1,33}=1{\cdot}11$), but the interaction of species × sex was close to significance ($F_{5,33}=2{\cdot}35$, $P=0{\cdot}063$). Thus it is probable that the difference between the sexes in total voracity varies according to species: in *A. javana*, *B. serarius*, *E. alternans* and *E. balteatus*, males have a greater total intake than females, whereas in *I. scutellaris* and *E.* (*M.*) *confrator* the opposite is true.

Are these differences caused by size differences? An analysis of covariance used final larval size as covariate: in this case, the regression (covariate) was not significant ($F_{1,32}=0{\cdot}47$). A further possibility is that the initial larval size on emergence from the egg is a determinant of the eventual total voracity. An analysis of covariance found a very significant regression ($F_{1,32}=6{\cdot}17$, $P<0{\cdot}01$). As before, even when we adjust for initial larval size, species differences remain ($F_{5,32}=7{\cdot}04$, $P<0{\cdot}001$). There are no sex differences ($F_{1,32}=0{\cdot}43$).

4. Discussion

These data suggest that several of the contrasts made were highly influenced by size, but that this failed to account for all differences between species. Few comparisons

between the sexes were significant, but we suspect this is more a reflection of low sample sizes than a true result.

The data further suggest that *E.* (*M.*) *confrator* is possibly a more useful predator than the other species, at least from the standpoint of the number of aphids killed per predator during development. Efforts to improve the biological control of *L. erysimi* using syrphids should concentrate on this species, to determine whether other aspects of its biology and ecology are also favourable for its use in biocontrol.

Opinion is divided as to the utility of syrphids as biocontrol agents of aphids. Work done 20 years ago seemed to write them off due to their lack of synchrony with their prey, and their inability to restrain aphid numbers below an economic threshold. More recent work (Chambers and Adams 1986) suggests that this view may be premature, and that particularly in cereals and in greenhouses but also elsewhere they may play a major role. It is certain that many previous studies relied on inadequate sampling methods: syrphids larvae are largely nocturnal feeders, moving off the plant during the day. Daytime sampling procedures adequate for aphids and other predators may fail completely to provide even roughly accurate density estimates.

Efforts to model the effect of aphid-specific predators have been hampered in the case of syrphids by a lack of appropriate data (Raworth 1984). A complete survey of

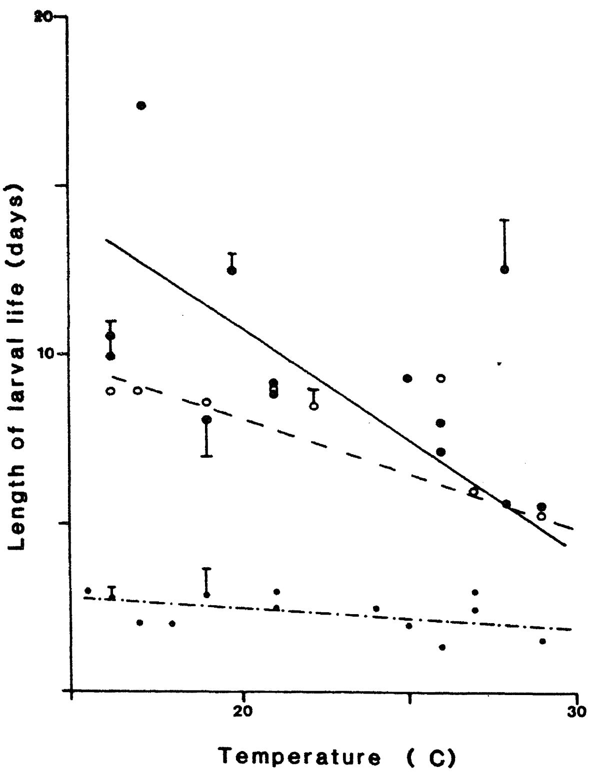

Figure 3. Literature data on longevity of the egg, larva and puparium of *I. scutellaris*. Data from Lal and Lal Gupta (1953), Lal and Haque (1955), Patel and Patel (1969), Mathur and Sharma (1973), Patnaik and Bhagat (1976), Gafarov (1979), Alfiler and Calilung (1980) and Agarwala and Saha (1986). (●), Larvae; (○), puparia; (○), eggs.

syrphid literature (Gilbert F S, unpublished results) actually reveals significant ›unts of usable data. As an example, we consider just one of our 6 species, *cutellaris*. Figure 3 plots literature data on longevity of the stages as a function temperature (uncontrolled in some studies, such as this one). There is a iificant negative relationship, as expected (e.g. larval longevity, $r = -0.53$, $n = 12$, 0·05). The data reiterate Benestad's (1970) conclusion that fluctuating peratures (such as in this study) have very nearly the same effect on longevity as onstant one equivalent to the mean: therefore laboratory data from constant peratures may well be sufficiently realistic to use in modelling. Such data as ours not unusual in the literature, and could prove valuable for use in population dels of aphid dynamics (models which of course will require validation from field lies).

)ut of the 6 syrphids studied, *E.* (*M.*) *confrator* shows the greatest predatory ential, at least from this laboratory study of voracity; the other 5 species are re or less equivalent in these terms. Future work will address the impact of ›hid predator on *L. erysimi* in the field, which we suspect to be substantial, to whether this prediction is in fact true. We believe that syrphid predators show at promise as elements in the biocontrol of *L. erysimi* in cruciferous crops.

:nowledgement

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Role of soil fauna in decomposition of rice and sorghum straw

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Abstract. This study examines the mass loss patterns and meso-invertebrate populations during rice and sorghum straw decomposition, using litter-bag technique, in an agricultural system at Kurukshetra (29°58′ N, 76°51′ E, 250 m above mean sea level). The decomposition rates were influenced by litter quality, soil and litter moisture, leaching of water soluble substances, and colonization by the meso-invertebrates. During the cropping season, wet soil conditions favoured rapid decomposition rates. For the total sampling period of 285 days, the mass loss of rice and sorghum straw was 78·2 and 82% respectively. The single exponential model described the pattern of decomposition over time ($r^2 = 0{\cdot}88$, $P < 0{\cdot}001$). Meso-invertebrate populations were higher during rapid phase of decomposition and influenced by soil and litter moisture. Maximum meso-invertebrate density per litter bag was $77{\cdot}4 \pm 6{\cdot}12$, for rice straw and $78{\cdot}4 \pm 3{\cdot}05$ for sorghum straw. Collembola and mites were the dominant groups of fauna in litter bags. Enchytraeids formed 2·45% of the total meso-invertebrates extracted from litter bags.

Keywords. Decomposition; straw; mass loss; meso-invertebrates; Collembola; mites; nitrogen; C:N ratio.

1. Introduction

Decomposition is an important functional process which regulates to a large extent energy flow and nutrient cycling in terrestrial ecosystem (Odum 1971). It is a complex and multistep process which is brought about by combined action of leaching, activity of soil animals and microorganisms. The soil animals constitute an important group of organisms involved in decomposition of organic debris in the soil. Many workers have studied the role of soil animals in litter decomposition in natural ecosystems (Edwards *et al* 1970; Edwards 1974; Lofty 1974; Mason 1974; Wood 1976; Anderson *et al* 1983; Seastedt 1984). In agricultural systems, the role of soil fauna in decomposition has been investigated by a few workers (Coleman *et al* 1984; House and Parmelee 1985; Coleman 1985). The objective of this study was to analyze the decomposition rates of sorghum and rice straw and the meso-invertebrates associated with decomposing litter.

2. Study area

The study was conducted in an agricultural system in a dry subhumid region at Kurukshetra (29°58′ N, 76°51′ E, 250 m above MSL). The soil of the area is alluvial which is sandy loam in texture and slightly calcareous in nature (Duggal 1970). Some physical and chemical characteristics of soil are given in table 1. The climate is tropical monsoon with 3 recognisable seasons in a year; rainy, winter and summer seasons (Singh and Yadava 1974). Monthly variations in temperature and rainfall during the study period from September 1986 to August 1987 are shown in

Table 1. Soil characteristics of the study site.

Soil property	
Soil texture	Clay loam
Bulk density (g cm^{-3})	1·35
Water holding capacity (%)	46–50
pH (1:2)	8·05–8·13
Organic carbon (%)	0·46–0·92
Nitrogen (%)	0·046–0·092
Phosphorus (%)	0·022–0·025

figure 1. Maximum temperature varied from 16·8–40·4°C and total rainfall was 150·6 mm.

3. Materials and methods

Bulk samples of sorghum and rice straw were collected from the agricultural field during September 1986 after crop harvest. The litter bags were prepared by placing 5 g chopped straw material separately into 2 mm mesh, 20 × 10 cm nylon wire netting cloth bags. The litter bags were placed at random (buried at 5 cm depth) in 2 × 0·5 m plots marked within an area of 20 × 25 m. The litter bags were set out in the field on 30 November 1986.

Four bags of each material type were recovered from the field after 15 days on the first sampling date and at one month interval on the subsequent dates up to September 1987. The material of one bag was used for determining the moisture content and other 3 bags were used for extracting meso-invertebrates from litter bags. The fauna from the litter bags was extracted by Burlese funnel method. The fauna were transferred into a jar containing water. The samples of fauna were then separated into different groups and their numbers were counted using a stereo-binocular microscope.

After the extraction of meso-invertebrates from litter bags, the material was washed under a fine jet of water. The washed material was soaked dry on blotting sheets and oven dried at 80°C. From the decrease in initial mass of litter in the bags, the decomposition rates were calculated.

The initial samples of sorghum and rice straw were analysed for phosphorus, calcium and magnesium following standard methods of Allen *et al* (1974). Total nitrogen was determined by the semi micro-Kjeldahl method. Ash content was determined by igniting 1 g oven-dried sample at 600°C in a muffle furnace for 5 h. Water soluble substances were estimated by leaching 1 g plant sample in 500 ml distilled water for 24 h. Organic carbon was analyzed by semi-micro carbon analyzer using combustion method.

A '*t*' test was used to compare mass loss and the populations of meso-invertebrates due to sampling dates and litter types. The per cent mass remaining in litter bags and populations of meso-invertebrates were related to the number of days using regression analysis following Sokal and Rohlf (1969). Correlations were also found between populations of meso-invertebrates and mass loss, litter moisture and soil moisture.

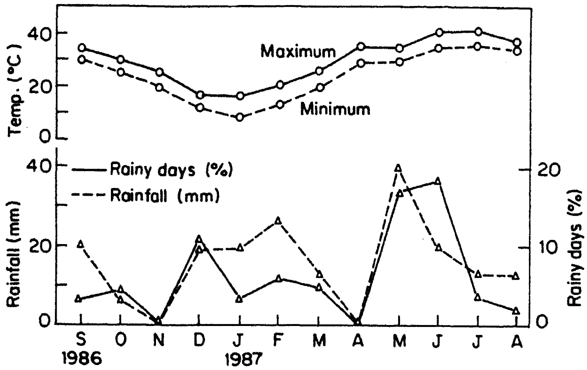

Figure 1. Monthly variations in temperature, rainfall and number of rainy days of the study area during September 1986 to August 1987.

To determine relative decomposition rates (for individual sampling dates), the following formula was used according to Anderson (1973) and Gupta and Singh (1981):

$$R = \frac{\ln W_1 - \ln W_0}{t_1 - t_0},$$

where R = mean relative decomposition rate (g/g/day), W_1 = litter mass at time t_1 and W_0 = litter mass at time t_0.

4. Results

4.1 *Litter decomposition*

The per cent chemical composition of sorghum and rice straw in terms of ash content, organic carbon, nitrogen, water soluble components (WSC) and some nutrient elements is given in table 2. Sorghum straw was characterized by high concentration of nitrogen, phosphorus and water soluble components and narrow C:N ratio (25:1). Rice straw represented a nutrient poor substrate with wide C:N ratio (59:1).

The variations in soil water and litter moisture during the observations of decomposition rates of straw materials are shown in figure 2. On the 4 initial sampling dates during 14 December 1986 to 25 February 1987, the soil moisture content (17·9–32·6%) and litter moisture (30·3–164·0%) were high. From March to September 1987, litter moisture fluctuated between 10·2–20·7% and the soil moisture content was low (7·3–15·4%). Rainfall during May and June caused slight increase in litter and soil moisture.

Per cent mass loss for sorghum and rice straw on various sampling dates is represented in figure 3. The decomposition rates of sorghum straw were higher than that of rice straw. The mass loss data of individual sampling dates were compared using 't' test, the differences were significant on 4 sampling dates, i.e. 30 December 1986, 30 January, 27 March and 22 July 1987 (t = 3·17–4·27, P < 0·05, d.f. = 4). The decomposition rates were high on first sampling date due to leaching of water

Table 2. Chemical composition of sorghum and rice straw used in decomposition experiment.

Chemical constituent (%)	Sorghum straw	Rice straw
Ash	15·0	14·9
Carbon	47·3	48·1
Nitrogen	1·87	0·81
Phosphorus	0·07	0·015
Calcium	0·48	0·53
Magnesium	0·12	0·22
Water soluble compounds	18·21	15·53
C:N ratio	25:1	59:1

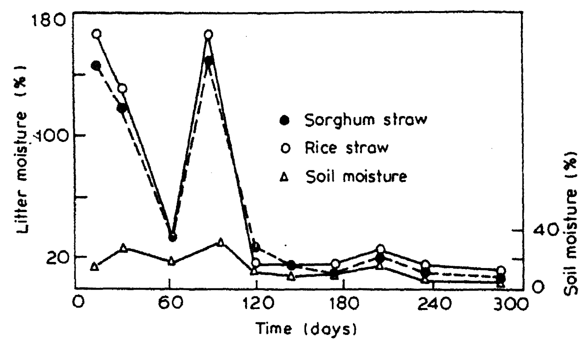

Figure 2. Variations in soil moisture and litter moisture of sorghum and rice during 30 November 1986 to 10 September 1987.

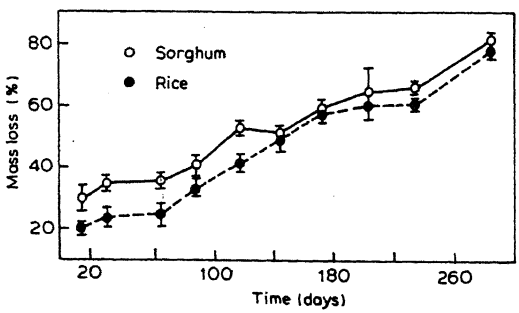

Figure 3. Per cent mass loss of sorghum and rice straw during 30 November 1986 to 10 September 1987.

soluble substances. The rates tended to be slow during cold winter months of December and January. Due to moderate temperature and moisture conditions in February, the decomposition rates increased up to May 1987. The low availability of moisture during July and August slowed down the activity of decomposer organisms resulting in slower rates of mass loss. During the total sampling period of 285 days, the mass loss of sorghum and rice straw was $82{\cdot}0 \pm 1{\cdot}58\%$ and $78{\cdot}21 \pm 1{\cdot}63\%$ respectively.

The mean relative decomposition rates during individual sampling dates are given in table 3. The relative decomposition rates were highest for the sampling interval 30 November to 14 December 1986. Subsequently, rates varied from 0·00107–0·0078 for sorghum straw and from 0·00026–0·0052 for rice straw. The rates were found to be high for the last sampling interval from 23 July to 10 September 1987 (table 3).

Soil and litter moisture were found to be important factors affecting decomposition rates, the rates being higher when soil was wet. The effect of soil and litter moisture was evaluated by correlating mean relative decomposition rates (RDR) of a sampling interval to its corresponding litter and soil moisture. There was no significant relationship between these abiotic variables and relative decomposition rates for both the straw materials ($r = 0{\cdot}26$–$0{\cdot}38$, d.f. $= 8$, $P > 0{\cdot}05$).

To determine temporal pattern of decomposition, regressions were fitted on the data of per cent mass remaining in litter bags over time. The relationship was significant and explained 88% variability in mass loss due to time. The regression equations are:

Sorghum straw:

$$\log_e y = 4{\cdot}41 - 0{\cdot}00469 \log_e X$$
$$(r^2 = 0{\cdot}88,\ \text{d.f.} = 28,\ P < 0{\cdot}01)$$

Rice straw:

$$\log_e y = 4{\cdot}52 - 0{\cdot}00434 \log_e X$$
$$(r^2 = 0{\cdot}89,\ \text{d.f.} = 28,\ P < 0{\cdot}001)$$

where y = per cent mass remaining in litter bags and X = time in days.

4.2 *Meso-invertebrates*

The colonization patterns of different meso-invertebrates during decomposition of

Table 3. Mean relative decomposition rates (g g^{-1} day^{-1}) of sorghum and rice straw during 10 sampling intervals from 30 November 1986 to 10 September 1987.

	Mean relative decomposition rate (g g^{-1} day^{-1})		
Sampling interval	No. of days	Sorghum straw	Rice straw
30 Nov.–14 Dec. 1986	15	0·0238	0·0149
15 Dec.–30 Dec.	16	0·0046	0·00269
31 Dec.–30 Jan. 1987	31	0·00373	0·001193
31 Jan.–25 Feb.	26	0·00326	0·00403
26 Feb.–27 March	30	0·00728	0·0042
28 March–22 April	26	0·00107	0·00573
23 April–22 May	30	0·01043	0·00626
23 May–22 June	31	0·00519	0·00514
23 June–22 July	30	0·0032	0·00026
23 July–10 Sept.	50	0·0115	0·0115
Total study period	285	0·0078	0·0052

Initial oven dry weight of rice straw = 4·850 g; sorghum straw = 4·880 g.

straw materials indicated that collembola and mites were the dominant group of fauna (tables 4 and 5). Meso-arthropod densities were initially low on the first 3 sampling dates during winter months. Collembola and mites exhibited their peak populations during February and March when both temperature and moisture were moderate. Due to dry conditions in summer months, the population showed a sharp decline. The availability of moisture during cropping season did not increase the density of fauna appreciably. It is evident from tables 4 and 5 that enchytraeids were present in rice straw on all the sampling dates, whereas in sorghum straw, enchytraeids were not recorded on initial dates. The other meso-invertebrates were represented by Coleoptera adults and larvae, Dipteran larvae and miscellaneous group of fauna having lesser number of species. Their density varied from 2·1–8·1 on different sampling dates.

The relationship between populations of meso-invertebrates from decomposing straw and time showed positive significant correlation for Collembola ($r = 0{\cdot}65$–$0{\cdot}68$, d.f. $= 28$, $P < 0{\cdot}001$), mites and enchytraeids on sorghum straw ($r = 0{\cdot}86$–$0{\cdot}61$,

Table 4. Meso-invertebrate population on decomposing sorghum straw (number per litter bag).

Date	Collembola	Mites	Enchytraeids	Others	Total
14 December 1986	12·6 ± 5·03	9·2 ± 2·64	—	6·2 ± 1·52	28·0
30 December	7·1 ± 1·0	7·3 ± 4·04	—	4·5 ± 1·15	18·9
30 January 1987	13·3 ± 4·11	16·1 ± 4·0	3·2 ± 1·53	3·2 ± 1·15	35·8
25 February	21·6 ± 1·52	48·3 ± 3·51	1·3 ± 1·52	7·2 ± 2·08	78·4
27 March	11·1 ± 1·70	33·6 ± 11·87	5·6 ± 3·21	2·1 ± 2·0	52·4
22 April	6·3 ± 4·16	7·1 ± 2·64	—	3·5 ± 0·57	16·9
22 May	3·3 ± 0·51	4·3 ± 3·05	2·6 ± 1·15	3·4 ± 1·52	13·6
22 June	—	2·3 ± 0·51	1·6 ± 0·57	6·2 ± 1·52	10·1
22 July	5·1 ± 0·5	4·3 ± 2·52	4·6 ± 1·52	4·2 ± 1·15	18·2
August	—	—	—	—	—
10 September	3·4 ± 0·51	2·6 ± 0·50	8·3 ± 2·88	6·1 ± 2·00	20·4
Mean over the sampling period	8·3	13·5	2·7	4·7	29·2

Table 5. Meso-invertebrate population on decomposing rice straw (number per litter bag).

Dates	Collembola	Mites	Enchytraeids	Others	Total
14 December 1986	10·0 ± 2·70	14·2 ± 2·08	4·3 ± 4·50	4·2 ± 1·52	32·7
30 December	4·3 ± 0·85	5·3 ± 2·30	6·3 ± 3·21	3·5 ± 0·57	19·4
30 January 1987	11·3 ± 4·68	7·3 ± 3·21	1·66 ± 2·88	4·5 ± 1·52	24·8
25 February	25·1 ± 5·50	37·6 ± 3·81	6·6 ± 2·08	8·1 ± 2·0	77·4
27 March	11·2 ± 3·74	31·6 ± 7·50	3·0 ± 1·732	4·2 ± 0·57	50·0
22 April	6·30 ± 2·0	9·3 ± 2·51	—	2·3 ± 0·57	17·9
22 May	3·3 ± 2·36	6·3 ± 3·21	2·2 ± 1·0	7·0 ± 1·73	18·8
22 June	—	1·6 ± 1·4	—	4·3 ± 1·15	5·9
22 July	—	4·0 ± 3·46	3·6 ± 2·08	6·1 ± 1·0	13·7
August	—	—	—	—	—
10 September	2·6 ± 0·50	5·0 ± 3·46	12·6 ± 5·85	5·3 ± 2·08	25·5
Mean over the sampling period	7·4	12·2	4·0	4·9	28·5

d.f. = 28, $P < 0.001$) and total fauna of both the materials ($r = 0.38$–0.46, d.f. = 28, $P < 0.001$). The relationship was not significant for other groups of fauna ($r = 0.04$–0.17, d.f. = 28, $P > 0.05$).

High populations of soil meso-invertebrates generally corresponded to high soil and litter moisture, the populations being higher when soil and litter moisture were high. For both rice and sorghum straw, the populations of Collembola showed significant positive correlation with soil moisture ($r = 0.65$–0.78, d.f. = 28, $P < 0.001$) and litter moisture ($r = 0.55$–0.56, d.f. = 28, $P < 0.001$). The populations of mites, enchytraeids and total fauna did not show a significant relationship with soil and litter moisture ($r = 0.01$–0.32, d.f. = 28, $P > 0.05$).

The Collembola extracted from the litter bags were represented by 4 families, i.e. Entomobryidae, Folsomidae, Poduridae and Sminthuridae. *Sierra indica* was the dominant collembolan species on both the straw materials. Oribatid mites (Cryptostigmata) were recorded on all the sampling dates from the straw bags having highest densities of *Schelroribates* sp. Meso-stigmatid and Metastigmatid mites were particularly abundant in later phases of decomposing straw.

Total meso-invertebrate numbers per gram of straw material attained peak values after 88 days of placement of litter bags (figure 4). The meso-invertebrate densities were low on initial sampling dates as well as during last phases of decomposition. On all the sampling dates, the number of fauna was higher on sorghum straw as compared to rice straw, however, the differences were not significant ($t = 0.01$–0.02, d.f. = 4, $P > 0.05$). Across the sampling dates, total number of meso-invertebrates averaged 12·54 and 14·26 individuals g^{-1} straw of rice and sorghum respectively. The populations of meso-invertebrates on decomposing straw showed higher values during rapid phases of decomposition. The population of Collembola and enchytraeids on sorghum straw showed significant relationship with average mass loss ($r = 0.67$–0.71, d.f. = 8, $P < 0.05$). Other groups of fauna did not show significant relationship ($r = 0.21$–0.56, d.f. = 8, $P > 0.05$).

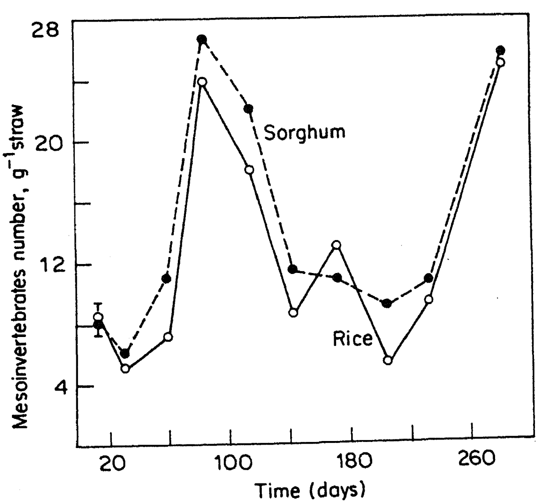

Figure 4. Total number of meso-invertebrates (No. g^{-1} straw) during decomposition of sorghum and rice straw on various sampling dates during 30 November 1986 to 10 September 1987.

5. Discussion

The litter decomposition rates were influenced by litter quality, soil and litter moisture, leaching of water soluble substances and colonization by soil invertebrates. The high mass loss of straw materials on initial sampling dates could be attributed to leaching of water soluble substances that occur in the presence or absence of microflora and fauna (Witkamp and Crossley 1966; Anderson 1973; Vossbrink *et al* 1979; Gupta and Singh 1981). Under field conditions, the mass loss due to leaching amounted to 15·9 and 14·3% for sorghum and rice straw respectively. Gupta and Singh (1981) studied the role of leaching in decomposition of herbaceous litter and showed that leaching losses varied from 25–76% depending upon the nature of the plant material. The higher decomposition rates of sorghum straw in the early phase could be due to its high nitrogen content and water soluble compounds compared to rice straw.

Relative decomposition rates indicate the effect of litter type, litter moisture and wetness of surface layers of soil. The wet soil conditions during cropping season showed higher rates of decomposition. Significant relationship between decomposition rates and time clearly defined the temporal pattern of decomposition of straw materials explaining 88% variability in decomposition rates which is in conformity with the results of Anderson (1973) and Gupta and Singh (1981).

Information on the functioning of soil invertebrates in decomposition of crop residues is limited (House and Stinner 1983; Coleman 1985; House and Parmelee 1985). These studies relate to the structure of soil arthropod communities in agricultural systems. The present study showed the importance of collembola and mites in litter decomposition as these formed 26–89% of total meso-fauna extracted from the decomposing straw on various sampling dates. The populations of meso-invertebrates on decomposing straw corresponded to decomposition rates; the populations being higher during rapid phases of decomposition. For sorghum straw, collembolan populations showed significant relationship with mass loss ($r = 0{\cdot}71$). Crossley (1977) discussed the role of soil fauna in decomposition in forest ecosystems and stated that soil arthropods are less abundant than soil microflora, and have slower metabolic and turnover rates. To a large extent, decomposition of plant debris is brought about by microbial activity in the soil (Singh and Gupta 1977). McBrayer *et al* (1974) reported that soil fauna contribute less than 1% to the annual CO_2 evolution from forest soils. Thus, soil fauna have indirect effects on decomposition by affecting the processes as conversion of litter to faeces, fragmentation of litter, mixing of litter and soil, regulation of microflora and reducing the immobilization of nutrients through senescent fungal tissues (Edwards *et al* 1970; Crossley 1977).

For evaluating the role of soil invertebrates in decomposition, Edwards and Heath (1963) used litter bags of different mesh sizes and found that *Quercus* and *Fagus* leaves disappeared 3 times as quickly from 7 mm mesh size bags compared to 0·003 and 0·5 mm mesh bags. Gupta and Singh (1977) reported higher mass loss of litter from coarse mesh bags (89·7%) than from the fine mesh bags (55·4%). They indicated that decomposition rates are high when all groups of soil fauna and microorganisms take part in decomposition. Santos *et al* (1981) found that exclusion of microarthropods from litter by insecticides and fungicides reduced decomposition by 50%. These studies in a gross way demonstrate the impact of soil fauna on decomposition.

In this study, it was found that detritivores like collembola, mites and enchytraeids played a significant role in decomposition of straw as the populations of meso-invertebrates were higher during rapid phases of decomposition. The nutrient rich substrate (sorghum straw) had higher populations of saprophagic fauna as compared to nutrient poor rice straw. Litter and soil moisture affected the mass loss and populations of saprophagic fauna.

Acknowledgement

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Mass loss and concentrations of nutrients in relation to microarthropod abundance, during needle decomposition in a pine plantation

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Abstract. Mass loss and concentrations of N, P, Ca and Mg of needle litter in relation to microarthropod population abundance during decomposition were investigated by the litter-bag method in a pine (*Pinus kesiya* Royle) plantation in north-eastern India. Collembola and Acarina together constituted 97·5% of the total microarthropods. Nitrogen slowly increased in its concentration in litter as decomposition progressed. Concentrations of Ca and Mg increased while that of K decreased in the litter. The microarthropods showed significant positive correlation with mass loss of the litter, but did not show any relationship with the N and Ca concentrations. Acarina abundance showed significant positive correlation with Mg concentrations of litter. The microarthropods other than Collembola and Acarina, however, showed significant negative correlation with K concentration of litter although they were recorded in meagre numbers.

Keywords. Microarthropods; needle litter; mass loss; nutrients; *Pinus kesiya* R.

1. Introduction

Mass loss of litter and its rate of mineralization during decomposition is significantly influenced by the abundance and activities of microarthropods (Swift *et al* 1979; Seastedt 1984). A number of studies have demonstrated that the soil fauna regulate these processes (Witkamp and Crossley 1966; Seastedt and Crossley 1980; Anderson *et al* 1983). While such reports are common from temperate regions, little information is available on the effect of fauna on decomposition and mineralisation in tropical ecosystems. Thus, the present investigation was undertaken to study the relationships between the abundance of microarthropods and the mass loss, and concentrations of some mineral nutrients of needle litter of pine (*Pinus kesiya* Royle ex-Gorden) during decomposition under field conditions.

2. Study area

The study area was a *P. kesiya* plantation of about 15 years old near Shillong (Lat. 25°34′ N, Long. 90°56′ E and altitude 1250 m above MSL, north-eastern India), which has been the location of an intensive study of the structure and function of this ecosystem since 1975. A detailed description of the area has been given in Reddy (1984). The climate is subtropical with monthly total rainfall, litter moisture and temperature ranging from 0–72·9 cm, 15–73·8% and 16·5–25·5°C respectively (figure 1). The rainy season extends from the end of March to October with maximum rainfall during June–July and minimum in February, winter being experienced from November to February and a mild summer in March.

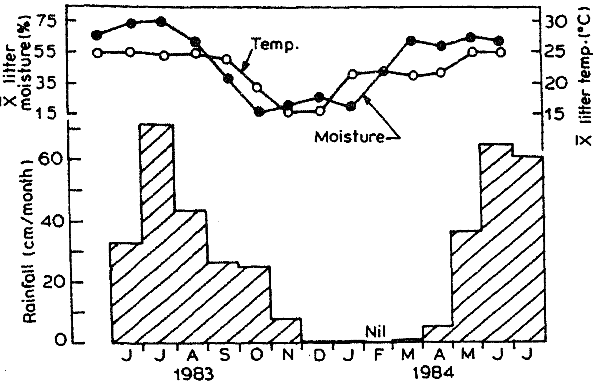

Figure 1. Seasonal changes in the total monthly rainfall, litter-moisture and litter-temperature at the plantation site.

3. Materials and methods

The abundance of litter inhabiting microarthropods and litter for the analysis of mineral nutrients such as N, K, Ca and Mg were sampled by the litter-bag method (Crossley and Hoglund 1962). The freshly shed pine needles were collected during the litter fall season from the plantation site, air-dried and cut into uniform size of 5 cm length. Ten g of the air dried litter was placed in 10 × 10 cm nylon mesh bags of 1 mm pore size. These litter filled bags were placed on the floor of the plantation and covered with a thin layer of freshly fallen needle litter. Three bags were collected from the field at monthly intervals beginning 30 days after placement of the bags on the plantation floor. Microarthropods were extracted with Tullgren funnels and were stored in 80% ethanol for identification and counting. The litter i.e. litter and associated microflora of the bags, was separated from adhering soil particles, oven dried at 75°C and weighed. Samples were then powdered and analysed for N, K, Ca and Mg following the methods of Allen *et al* (1974). Rate of mass loss was measured as loss in oven-dry weight (Curry 1969).

Data were analysed by a stepwise selective multiple regression model to assess the relationships between the population abundance of different groups of microarthropods, and mass loss and concentrations of the nutrients of the decomposed litter.

4. Results and discussion

The abundance of microarthropods per g of litter of the litter-bags varied considerably and was influenced by the abiotic factors, particularly rainfall (figure 2; Reddy 1984). Collembola comprised 67·5% of the total fauna, with *Isotoma trispinata* MacGillvray being dominant. The Acarina comprised 30% of the fauna and were dominated by a cryptostigmatid, *Galumna* sp. Miscellaneous groups such as Pauropoda, Diplopoda, Symphyla, Hymenoptera, Coleoptera, Araneida and Dipteran larvae constituted the remaining 2·5%.

During August 1983 and January 1984 when the populations of microarthropods were very low, the mass loss of litter from the litter-bags was also low. In contrast,

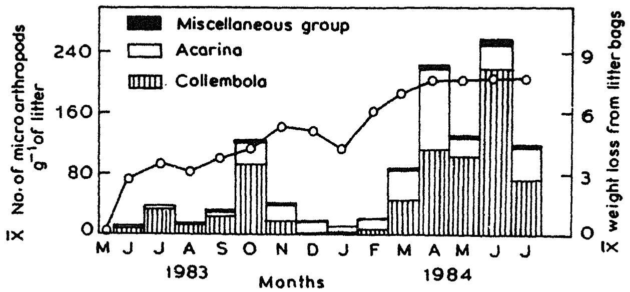

Figure 2. Seasonal abundance of microarthropods per g of the litter and mean loss of litter mass.

during the last 4 months of the investigation when the microarthropod populations were relatively more dense, the mass loss was maximum (figure 2). The fragmentation activities of the microarthropods in the litter was evident from the disintegrated litter recovered from the litter bags. Macroinvertebrate activity in the bags was minimal due to the mesh size of the litter bags. Collembola, Acarina and miscellaneous taxa were all positively correlated with mass loss (table 1). The microarthropods along with abiotic factors such as rainfall, litter moisture and temperature showed significant combined influence on the mass loss of litter (multiple regression: $R^2 = 0{\cdot}75$; $P < 0{\cdot}05$). The mass loss in May–June was probably attributable to leaching of soluble organic compounds (Swift *et al* 1979). Decay after one year was about 80% of the litter, a value much higher than those reported for temperate zones (Cromack and Monk 1975; Fogel and Cromack 1977), and was most probably due to the combination of higher faunal densities in conjunction with greater microbial activity.

Seasonal patterns of N, K, Ca and Mg concentrations in litter showed much less seasonal variation than would be expected from the bimodal pattern of rainfall (figure 3). Nitrogen concentrations slowly increased throughout the first year of decay while Mg exhibited no clear pattern. Calcium exhibited a sharp decline during the initiation of the dry period, while K showed an increase in concentrations during the dry periods. This increase is correlated with an apparent absolute increase in litter mass (figure 2), suggesting dry particulate deposition on the litter (Seastedt and Crossley 1980). Nutrient loss from decomposing litter is the net effect of changes in nutrient concentrations and litter mass. The general absence of changes in nutrient concentrations relative to the densities of fauna (table 1 and figure 2) indicates that intense periods of microbial immobilization followed by rapid pulses of mineralisation do not occur. Given that the densities of fauna are generally high per g of litter [often over two to four times those reported by Seastedt *et al* (1983) for southern appalachian litter of similar age], fragmentation and grazing effects appear to be the dominant factors influencing elemental dynamics. This would explain the strong correlations with monthly mass loss, but few correlations with nutrient concentrations of decaying litter.

Although microarthropods have often been reported to influence the net mineralisation of the chemical elements of litter (Seastedt 1984), there were no significant correlations between N and Ca concentrations of the decomposed needle litter and the abundance of any of the groups of microarthropods (table 1). The lack

Table 1. Regression equations between the abundances of different groups of microarthropods, and the mass loss and concentration of N, K, Ca and Mg (% of dry mass) of the decomposed needle litter.

Different arthropod taxa	Loss in litter mass	N	K	Ca	Mg
Collembola	$Y = 5{\cdot}58 + 0{\cdot}007X$ $t = 2{\cdot}05^*$	—	—	—	—
Acarina	$Y = 5{\cdot}9 + 0{\cdot}02X$ $t = 2{\cdot}9^{**}$	—	—	—	$Y = 0{\cdot}2 + 0{\cdot}004X$ $t = 2{\cdot}08^*$
Miscellaneous	$Y = 6{\cdot}17 + 0{\cdot}28X$ $t = 4{\cdot}0^{**}$	—	$Y = 0{\cdot}07 - 0{\cdot}014X$ $t = 2{\cdot}19^{**}$	—	—

Level of significance: $^*P < 0{\cdot}05$; $^{**}P < 0{\cdot}01$.

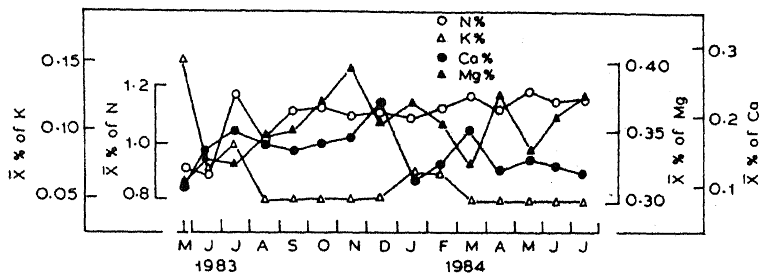

Figure 3. Seasonal changes in the mean concentration of N, P, Ca and Mg (% of dry mass) of the decomposed litter.

of a relationship supports the findings of Macauley (1975) that microarthropods have little effect on the nitrogen concentrations of the decomposed litter. However, Anderson *et al* (1983) reported that Collembola have a considerably great effect on the loss of N from the decomposed litter than any other taxa of soil fauna. Similarly, these microarthropods may have little effect on the amount of Ca (Seastedt 1984). However, Ineson *et al* (1982) reported that grazing of Collembola significantly increased the leaching of ammonium, nitrate and Ca from decomposed oak litter. The K concentration of the decomposed litter, while not showing any correlation with the abundance of Collembola and Acarina, was significantly negatively correlated with the abundance of other (miscellaneous) microarthropods (table 1). This group, although few in number, may facilitate the leaching of K from litter. However, Ineson *et al* (1982) reported that this element was little affected by microarthropods. The Mg concentration of litter showed significant positive correlation with the abundance of Acarina in litter bags (table 1), but there was no correlation between its concentration and the abundance of Collembola and the miscellaneous microarthropods. Seastedt (1984) reported that microarthropods have no consistent effect on Mg of the decomposed litter. Overall, this study suggests that microarthropods played a significant role in the mass loss of the decaying needle litter while having very little effect of nutrient concentrations. The net result of rapid mass loss with no significant increase in elemental concentrations of this decaying mass implies very rapid mineralization effects of tropical fauna.

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Morphometrics, micromorphology, microanatomy and cytochemistry of the gills of a swamp catfish, *Chaca chaca* (Ham.)

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Abstract. Dimensions, micromorphology, microanatomy and cytochemistry of the gills of a swamp catfish, *Chaca chaca* have been investigated using morphometric, scanning electron microscopic, light microscopic and cytochemical techniques respectively. The findings have been correlated with the respiratory efficacy of the gill sieve and the physico-chemical behaviour of the habitat of the fish.

The filament number among 4 gill arches varies from 144–151 and does not show any significant ($P > 0{\cdot}05$) difference among themselves. The filament length shows heterogeneity in their relative dimensions at different points along the various hemibranchs of the 4 gills. Lamellar frequency (n) and area (bl) were 31·2 and 0·08316 mm^2 respectively. The small gill area (13905·3 mm^2) assigns comparatively low level of activity to the catfish. Total lamellar water flow of the gill sieve of *Chaca* was 20·123 cc/s.

The heterogenous gill arch epithelium contained taste buds and mucous gland openings. Short and stumpy gill rakers signify carnivorous feeding habit of the catfish. The micro-pockets of the gill filament epithelium entrap some water molecules to protect the gills from desiccation, when the fish is temporarily exposed to water-land interface and acts as a regular source of oxygen to the fish during adverse ecological conditions.

Keywords. Swamp catfish; morphometrics; micromorphology; microanatomy; cytochemistry; gills.

1. Introduction

Chaca chaca is a curious-looking catfish of the swamps of Kosi region (Bihar). This region is full of swamps infested with macrovegetation (*Vallisneria spiralis*). Such water bodies are the natural habitat of air-breathing fishes. During monsoon (June–September) and winter (October–February) seasons this region is submerged under normoxic water column. However, during summer the ambient water becomes hypoxic (DO_2–2 mg O_2/l). *Chaca* shares such an interesting habitat with the obligate air-breathing swamp-eel, *Monopterus cuchia*. The latter withstands the hypoxic condition of the swamp with the help of well-organised air-breathing organs (Munshi 1985) while, the swamp catfish does not have such organised air-breathing organs.

Fish gills are complex and are suited for gaseous and ionic exchange in extreme conditions of their habitat. The limnological characteristics of the habitat are responsible for various modifications in functional organisation of fish gills (Hughes 1984). The respiratory efficacy of gills is directly proportional to their effective respiratory area. In recent years various sampling procedures have been attempted for estimating the gill area of fishes inhabiting different ecological niches (Hughes 1984; Hughes and Ojha 1985; Ojha and Singh 1986). However, little is known about the lamellar flow during gill ventilation (Ojha and Singh 1986; Ojha and Hughes

Table 1. Various gill parameters of all arches from 100 g specimen of *C. chaca*.

Gill parameters	1st Arch Anterior hemibranch	1st Arch Posterior hemibranch	2nd Arch Anterior hemibranch	2nd Arch Posterior hemibranch	3rd Arch Anterior hemibranch	3rd Arch Posterior hemibranch	4th Arch Anterior hemibranch	4th Arch Posterior hemibranch	Total or mean two sides of fish
No. of filaments	71	73	74	75	73	74	77	74	1182
Average filament length (mm)	4·277	4·288	4·454	4·440	5·208	4·568	4·797	4·243	4·536
Total filament length (mm)	303·642	313·024	329·596	333·000	380·175	337·995	369·353	313·969	5361·388
Secondary lamellae/mm (both sides)	31·244		31·305		31·132		31·082		31·188
Total no. of secondary lamellae	9486·991	9779·372	10316·875	10424·565	11835·608	10522·460	11480·229	9758·789	167210·960
Average bilateral secondary lamellar area (mm^2)	0·08409		0·07224		0·08868		0·08718		0·08316
Gill area/mm filament (mm^2)	2·627		2·261		2·761		2·710		2·594
Total gill area/hemibranch (mm^2)	797·667	822·251	745·135	752·913	1049·663	933·204	1000·947	850·856	13905·271
Total gill area/arch (mm^2)	1619·918		1498·048		1982·867		1851·803		13905·271

Total gill area/arch both sides of fish	3239·836	2996·096	3965·734	3703·606	13905·271
Total gill area/arch both sides, if 1st arch sampled and % of error	3239·836	3481·090 (16·18%)	3773·265 (4·85%)	3590·174 (3·06%)	14084·365 (1·29%)
Total gill area/arch both sides, if 2nd arch sampled and % of error	2788·455 (15·34%)	2996·096	3247·565 (18%)	3089·982 (16·568%)	12122·098 (12·8%)
Total gill area/arch both sides if 3rd arch sampled and % of error	3405·0971 (5·1%)	3658·6563 (22·119%)	3965·734	3773·304 (1·88%)	14802·79 (6·4%)
Total gill area/arch both sides if 4th arch sampled and % of error	3342·1996 (3·159%)	3591·075 (19·85%)	3892·481 (1·84%)	3703·603	14529·358 (4·5%)

Values for expected total areas for both sides of the fish and % error, if only one arch had been sampled for measurements of secondary lamellar frequency and area.

middle and the upper zones. The middle zone is characterized by the presence of groups of sparsely distributed taste buds and the absence of mucous gland openings (figures 3, 4). Each taste bud is a dome-shaped structure with an opening at its apical part. The epithelium of the main body of the taste bud is thrown into girdle-like structures. Mucous gland openings are present in the apical and basal part of the gill arch epithelium.

The gill filament epithelium is thrown into many vertical furrows and elevations. The leading edge of the gill filament contains round sensory structures (figures 7, 8).

Well developed secondary lamellae originate from both sides of the gill filaments (figure 5). Each lamella is wider towards its leading edge than trailing edge (figure 6). The lamellar epithelium is thrown into numerous furrows (figures 9, 10).

3.4 *Microanatomy and cytochemistry*

Histological preparations indicate the presence of sparsely distributed mucous cells and taste buds while the gill filament epithelium show numerous mucous glands, the lamellar epithelium lack them (figures 13–15, 17). Red and blue mucous glands were differentiated by AB/PAS technique. Some mucous glands showed shades of red and blue granules (figure 15). Histological preparations also showed trematode cysts in the gill filaments (figure 16).

4. Discussion

4.1 *Gill dimensions*

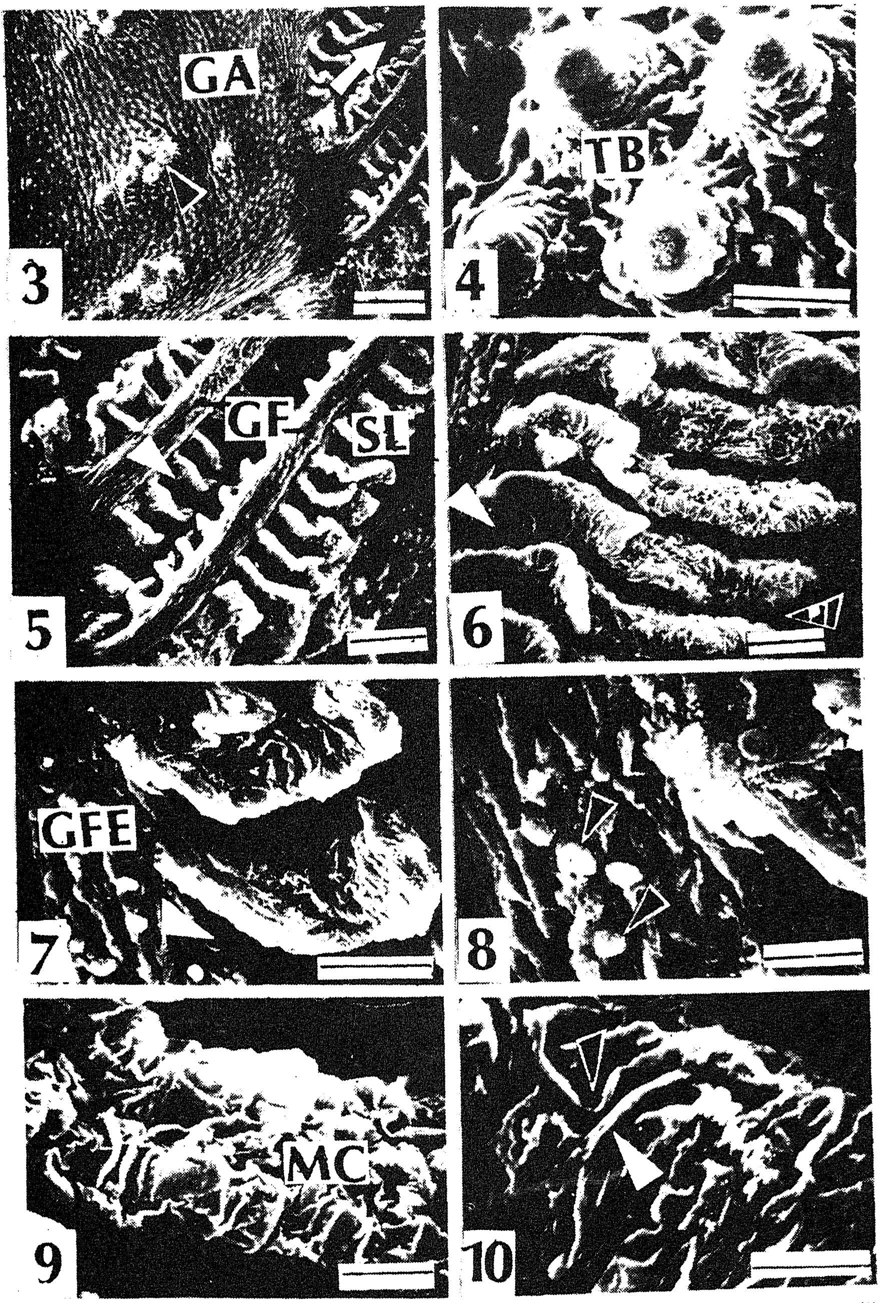
C. chaca inhabits swamps of Kosi region infested with macrovegetation. The gills of these catfishes are modified to suit the turbid flood waters during monsoon and hypoxic waters during summer. Interesting relationships are achieved when the data on different gill parameters are compared to those of fish of varied activities (table 3).

The density and distribution of gill filaments on different arches of *Chaca* reflect fairly homogenous nature of the gills. However, variations in the relative filament length on the two hemibranchs help to design a complete gill sieve to sample water for gaseous exchange.

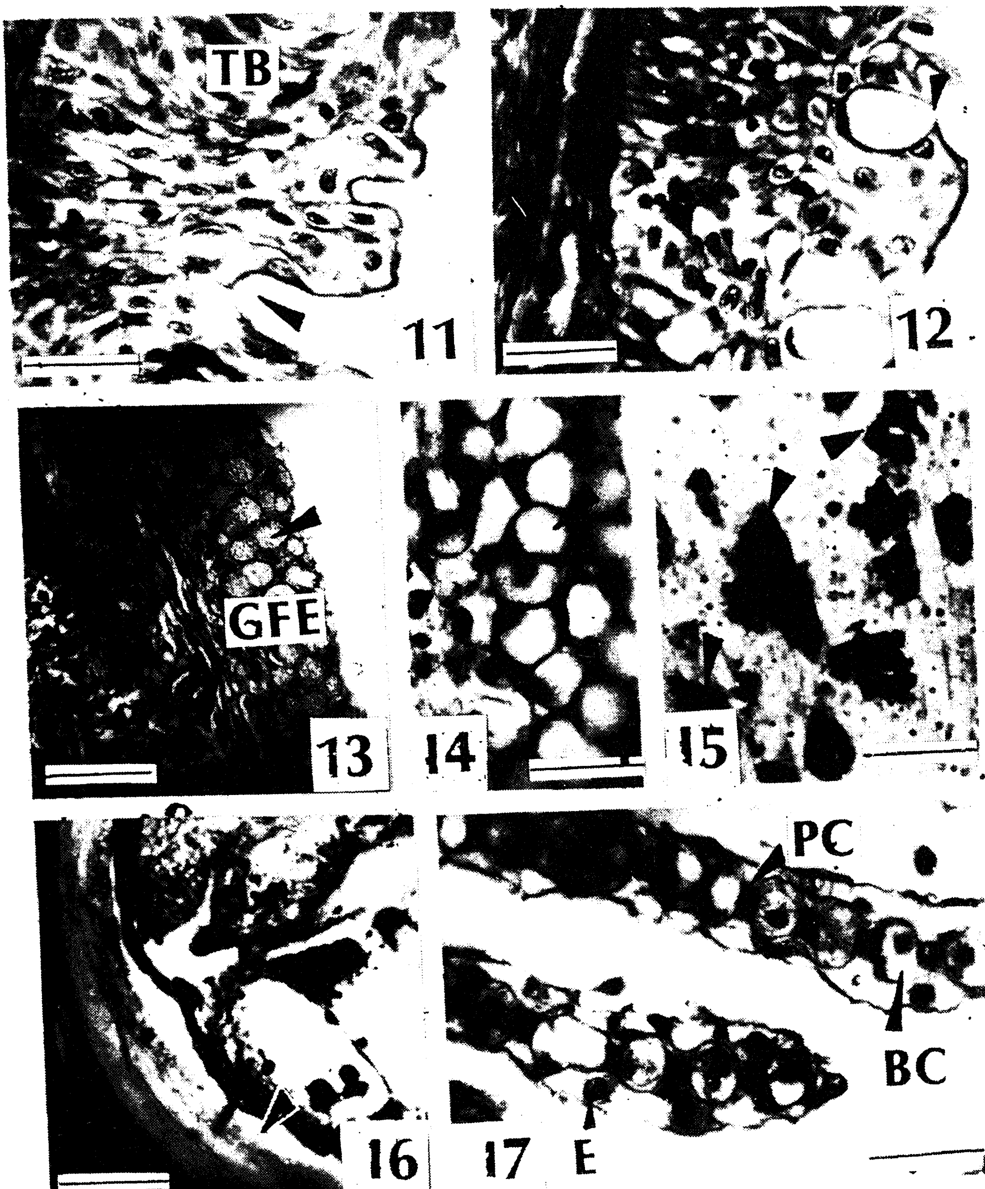
The filament length value falls below that of the riverine carp, *Catla catla.* Such smaller filament length assigns low level of activity to *Chaca.*

Lamellar frequency is an important gill parameter related to branchial water flow and gill sieve resistance. Lamellar frequency of *Chaca* is lower than those of active tuna, riverine carp and the air-breathing perch (table 3). A lower lamellar frequency obviously reflects wider inter lamellar space which in turn reduces gill resistance. Low water resistance is an adaptation to gill organisation of fish inhabiting turbid waters of the swamps and derelict ponds. Such gill sieve with low water flow resistance will allow smooth passage of turbid waters during gill ventilation and minimizes the chances of lamellar damage.

The average bilateral lamellar area (mm^2) of *Chaca* is large (0·083) and close to the value (0·1093) for *Anabas* (Hughes and Ojha 1985) inhabiting similar habital conditions. Such approximation in the lamellar structure in the two species is due



Figures 3–10. Gill of *Chaca chaca* examined by SEM. **3.** Central zone of the gill arch (GA) showing taste buds (small arrow). Interfilamentar space (arrow) is also discernible (bar = 240 μm). **4.** A part of gill arch epithelium showing taste buds (TB) (bar = 40 μm). **5.** Two gill filaments (GF) showing arrangement of secondary lamellae (SL) (bar = 110 μm). **6.** A part of gill filament showing its leading (arrow) and trailing (broken arrow) edges (bar = 60 μm). **7.** Rough gill filament epithelium (GFE) showing origin of secondary lamellae (arrow) (bar = 40 μm). **8.** Gill filament epithelium showing chemical sensors (arrow) (bar = 18 μm). **9.** A part of lamella showing marginal channel (MC) (bar = 16 μm). **10.** Lamellar epithelium (LE) showing elevations and grooves (arrow) (bar = 11 μm).



Figures 11–17. Gill of *Chaca chaca* examined by semithin sectioning. **11.** Gill arch epithelium showing mucous cells (arrow) and taste buds (TB) (bar = 20 μm). **12.** A part of gill filament showing mucous cell openings (bar = 20 μm). **13.** Gill filament epithelium (GFE) showing numerous mucous glands (arrow) [bar = 50 μm (**13**) and 20 μm (**14**)]. **15.** Gill filament epithelium showing mucous glands (AB–PAS) (bar = 20 μm). **16.** Parasitic cyst in the gill filament showing fibrous tissue around it (bar = 20 μm). **17.** Vertical section of lamellae showing blood channels (BC), epithelium (E) and pillar cells (PC) (bar = 20 μm).

Table 3. Various gill parameters of different fish species of 100 g body weight from varied habitat and activity.

Gill parameters	*Katsuwonus pelamis* (skipjack tuna) active-marine (Muir and Hughes 1969)	*Catla catla* (riverine carp) active, fresh-water (Hughes and Ojha 1985)	*Anabas testudineus* air-breathing perch, swamp (Hughes and Ojha 1985)	*C. chaca* catfish sluggish, swamp (Present work)
Total filament length (mm)	33498·1	20949·4	1422·1	5361·4
Lamellar frequency (2/d′)	83·2	54·2	41·7	31·2
Lamellar area (mm^2)	0·0940	0·0251	0·1093	0·08316
Gill area/mm (mm^2)	7·8208	1·3604	4·5578	2·594
Total gill area (mm^2)	261981·9	28499·6	6481·6	13907·5

to their convergent adaptation to similar habitat. Large secondary lamellae seem to be a compensatory device of nature to bridge the loss of respiratory area due to sparsely distributed lamellae on the gill filaments. Such an assumption is confirmed by evaluating the data on gill area per unit filament length which is the product of secondary lamellae per mm and average bilateral surface area of a secondary lamella. The denser turbid waters of the swamp also add to the buoyancy, and larger lamellae float freely in ventilating water of the gill sieve. However, densely packed larger lamellae in free-swimming fish overlap and disturb the normal functioning of the gill sieve including lamellar flow, gill resistance, gas exchange and energy cost during gill ventilation. Chances of such anarchy in active fish are eliminated by small lamellae, densely packed on the gill filament. The gills of the free-swimming river carp, *Catla catla* present such structural adaptations with densely packed (54/mm) smaller (0·025 mm^2) lamellae (table 3).

Total gill area (mm^2) of *Chaca* (13905·3) falls between those of the active riverine carp, *Catla* (28499·6) and the true swamp air-breathing perch, *Anabas* (6481·6) (Hughes and Ojha 1985). Such smaller gill area of *Chaca* is obviously due to its sluggish bottom dwelling habit in the swamps. *Chaca* cannot afford further reduction in the gill area due to the lack of true air-breathing organs. However, persistant oxygen demand because of smaller gill area may be met by cutaneous respiration.

It is interesting to note that in *Chaca* the sampling of the first gill for frequency and area of lamellae introduces least error (1·3%) and therefore it is suggested that for further studies sampling of only first gill alone be made to obtain good estimate of the gill area with least effort.

4.2 *Lamellar flow*

In addition to the dimensions, lamellar flow is also an important physiological parameter which influences the respiratory efficacy of the gills. The lamellar flow is directly proportional to the average height and interlamellar space and inversely proportional to the length of secondary lamellae. This suggests that the lamellar

dimensions influence the total gill area and also the lamellar flow. Because of the homogenous nature of the gill sieve the lamellar water flow does not differ significantly ($P > 0{\cdot}05$) in the 4 gills. Total lamellar flow of *Chaca* (20·123 ml/s) approximates the value (20 ml/s) reported for *Callionymus lyra* (Hughes 1966). Higher lamellar flow through per mm of the gill sieve of *Chaca* ($1{\cdot}758 \times 10^{-3}$ cc/s) than those of the hill-stream fish, *Danio dangila* ($1{\cdot}07 \times 10^{-5}$ cc/s, Ojha and Singh 1986) and the freshwater catfish, *Rita rita* ($4{\cdot}3 \times 10^{-5}$ cc/s, Ojha and Hughes 1988) is obviously due to higher values for interlamellar space and height of the secondary lamellae. Higher lamellar flow also indicates lower lamellar resistance to ventilating water across the gill sieve.

4.3 *Micromorphology*

The various delicate gill units of *Chaca* are modified to suit the adverse ecological conditions of the swamps. Small and stumpy gill rakers signify the carnivorous feeding habit of the fish. The taste buds on the gill arch take care of the chemical detection of the food present in ventilating water. The presence of taste buds in the central zone of the gill arch indicates heterogeneity in the gill arch epithelium. The absence of mucous gland openings in the middle zone is to protect taste buds from mucus covering which otherwise will affect their sensory efficacy.

The furrows and elevations of the gill filament epithelium trap some water molecules which protect gill filaments from desiccation when the fish is exposed temporarily out of water during summer. Sensory structures on gill filament epithelium are interesting and act perhaps as hydrochemical sensors, which have developed in response to adverse chemical nature of the swamp. Sensory structures are also reported from the gill filament epithelium of *Danio dangila* inhabiting hill-streams (Ojha and Singh 1986).

Well developed secondary lamellae with grooves and elevations add to the total effective respiratory surface for gaseous exchange. Such micropockets entrap some water molecules which protect lamellae from desiccation and help in respiration during adverse environmental conditions. During summer when the habital water dries up, *Chaca* prefers to move towards the water-land interface of the swamp and ventilate their gills with air which in turn oxygenates the water molecules entrapped in the lamellar epithelial grooves. Such oxygen-rich medium unloads its O_2 to the blood in the lamellar channels and offers supplementary source of oxygen to the catfish when it is exposed to air. This small amount of oxygen is just enough to sustain the catfish out of water. Encircling water around the trunk prevents dehydration and desiccation of the fish.

4.4 *Microanatomy and cytochemistry*

Fibrous covering around a mass of extra branchial tissue suggests pathological cysts and not the tumours. Formation of protective layer around parasites is a defensive mechanism of the organism. The presence of parasitic cysts in the gills suggest poor quality of the ambient water of the habitat in which *Chaca* lives and flourishes.

Mucous glands with blue and red stains by AB/PAS technique suggest

respectively acid and neutral glycoproteins secreted by the branchial glands. Such findings suggest heterogeneity in the chemical behaviour of the mucous glands of the fish. Few mucous glands, with a mixture of red and blue granules are suggestive of the stage of their transformation. From such findings it may be inferred that the same set of mucous glands can function differently under different ecological conditions of the habitat. However, the interaction between limnological behaviour of the habitat and the physiological state of the branchial glands of fish necessitates further studies.

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Impact of mating on the post-embryonic development of *Coranus soosaii* Ambrose and Vennison (Insecta—Heteroptera—Reduviidae)

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Abstract. Post-embryonic development of assassin bug *Coranus soosaii* Ambrose and Vennison was studied in the offsprings of 4 categories of females, viz. (i) virgins, (ii) mated once with a male of the same age, (iii) lived continuously with a male of the same age and (iv) lived with 3–4 males of different ages. Mating shortened the pre-oviposition period, enhanced the fecundity and hatchability, hastened the emergence and extended the adult longevity.

Keywords. *Coranus soosaii*; mating; oviposition pattern; hatchability; development.

1. Introduction

Reduviidae constitute an important group of predatory insects of southern India. The nutritional and reproductive adaptations of reduviids have reached a high degree of precision and efficiency (Ambrose 1980, 1987). Many of the reduviids are potential predators of many insect pests (Ambrose 1985, 1988).

Coranus soosaii is found to predate on insect pests like *Odontotermes obesus* Rambur, *O. assumthi* Holmgren and *Chrotogonus* sp. Efforts are being made to mass rear this bug and to use it as a biological control agent against various insect pests. Therefore detailed studies on the bioecology, ecophysiology and ethology of this bug are essential.

The effect of mating on oviposition pattern and hatchability has been studied in several species of insects (Riegert 1965; Loher and Edson 1973; Bentur and Mathad 1975; Subramaniam *et al* 1988). Among Heteropterans, mating influenced the size of egg batches, shortened the preoviposition period and enhanced the rate of oocytes development (Lee 1954; Ryckman 1958; Odhiambo 1968; Odhiambo and Arora 1973; Gorden and Bandal 1967). Similar observations were reported in reduviids, such as, *Acanthaspis pedestris* Stål, *A. siva* Distant, *Rhinocoris marginatus* Fabricius, *R. kumarii* Ambrose and Livingstone (Ambrose and Livingstone 1979, 1985a, b, 1987) and *R. fuscipes* Fabricius (Vennison and Ambrose 1986). However, no data are available on the influence of mating of the post-embryonic development of any reduviid. Hence, the present investigation is aimed at studying the effect of mating on oviposition pattern hatchability and nymphal development of *C. soosaii*.

2. Materials and methods

C. soosaii Ambrose and Vennison (Ambrose and Vennison 1989) is a harpactorine reduviid found in the scrub jungles, semiarid zones and tropical rain forests of Tamil Nadu. Gravid females were collected from the Maruthuvazhmalai scrub

jungle (latitude, 77°, 50′ E and 8°, 7 N), a legendary hillock in Tamil Nadu. They were maintained in plastic containers (220 ml) in the laboratory (32°C, RH 75–85%, photoperiod 11–12 h) on housefly (*Musca domestica* Linnaeus), black ant (*Camponotus compressus* Fabricius) and grasshopper (*Chrotogonus* sp.). The mating behaviour was studied in sex-starved individuals. The spermatophore index was calculated by using the following formula proposed by Ambrose (1980).

$$SI=\frac{LS}{LI}+\frac{WS}{WI},$$

where LI = length of male insect, LS = length of spermatophore, SI = spermatophore index, WI = width of male insect and WS = width of spermatophore.

To study the impact of mating on oviposition pattern and hatchability, adult females were kept under the following conditions: (i) no males (virgins), (ii) mated once with a male of the same age, (iii) lived continuously with a male of the same age and (iv) lived with 3–4 males of different ages. The containers were examined daily for the ejected spermatophore capsule that resulted from successful mating. The number of batches of eggs and number of eggs in each batch were recorded and each batch of eggs was allowed to hatch in an individual containers under optimum incubating conditions (temperature 30–35°C, RH 75–85%). Hatching percentage of each batch of egg and the longevity of the females were recorded. Indices of oviposition days (the percentage of egg laying days in total adult life of the female) were calculated (Ambrose 1980).

The laboratory-hatched nymphs of (ii), (iii) and (iv) categories were used to study the impact of mating on stadial period. Biological parameters like nymphal mortality, nymphal growth, sex-ratio and adult longevity were worked out individually and compared. All the experiments mentioned here were adequately replicated and statistically analysed.

3. Results

The longest pre-oviposition period ($9{\cdot}25 \pm 0{\cdot}5$ days) was observed for virgins (i). Among the mated females, the shortest preoviposition period (7 ± 1) was observed in group (iv). The influence of mating on the preoviposition period was highly significant ($P = 0{\cdot}05$) (table 1).

Mating also influenced the fecundity of *C. soosaii*. The group (iv) females laid the highest number of eggs ($74{\cdot}5 \pm 14{\cdot}87$) with highest number of batches of eggs (28). The (iii) and (ii) group females laid $45{\cdot}5 \pm 18{\cdot}64$ eggs in $16{\cdot}25 \pm 7{\cdot}76$ batches and $43{\cdot}75 \pm 11{\cdot}67$ eggs in $27{\cdot}25 \pm 12{\cdot}04$ batches respectively (table 1).

All the 4 categories of test individuals laid atleast one egg per batch. Virgins laid a maximum of $3{\cdot}25 \pm 0{\cdot}5$ eggs per batch which was comparatively lower than the maximum number of eggs per batch deposited by the mated categories, i.e. 5·5–5·67. Though the maximum number of eggs per batch laid by mated categories was significantly higher than that of the virgins, no significant variation was observed among the mated categories.

The highest oviposition index ($75{\cdot}67 \pm 8{\cdot}73$) was observed in the group (iv) females followed by the females of groups (ii) and (iii) ($53{\cdot}57 \pm 6{\cdot}28$ and $55{\cdot}94 \pm 6{\cdot}28$). The lowest oviposition index was recorded for virgins (i) ($48{\cdot}96 \pm 8{\cdot}06$).

The eggs laid by the females of (iii) and (iv) groups hatched after $8{\cdot}5 \pm 1{\cdot}29$ and

Table 1. Impact of mating on oviposition pattern in *Coranus soosaii*, n=6, ±SD.

	Categories of females			
Parameters	(i)	(ii)	(iii)	(iv)
Pre-oviposition days	9·25±0·5	9·25*±1·5	8·5±1·29	7·0**±1·0
Number of eggs	27·5±6·45	43·75**±11·67	45·5*±18·64	74·5±14·87
Number of batches of eggs	16·5±1·29	27·25±12·04	16·25±7·76	28·0±0·0
Minimum number of eggs per batch	1·0	1·0	1·0	1·0
Maximum number of eggs per batch	3·25±0·5	5·5*±1·73	5·5*±1·73	5·67*±2·31
Oviposition index	48·96±8·06	53·57*±6·28	55·94*±4·22	75·67*±8·73

Significance shown at $^*P<0{\cdot}1$; $^{**}P<0{\cdot}05$.
(i) No males (virgin); (ii) mated once with a male of the same age; (iii) lived continuously with a male of the same age; (iv) lived with 3–4 males of different ages.

9·25±1·5 days respectively. The eggs of group (ii) females had the shortest incubation period, and the eggs of group (iv) had the longest incubation period. Mating extended the incubation period in *C. soosaii* (table 2).

Eggs laid by virgin females did not hatch. The highest number of nymphs hatched from the eggs of the group (iv) females followed by the eggs of group (iii) females. The group (iii) females laid the lowest number of viable eggs. The hatching percentage was highest among the females of group (iv). Thus, mating had a positive proportionate impact on the hatchability of *C. soosaii* (table 2). The data further indicated that the older females laid more nonviable eggs than the younger females.

The shortest first stadium (8·9±1·92) was observed in the offsprings of (ii) group females. The offsprings of (iv) group females had the shortest second stadium (6·46±0·9) while the (ii) and (iii) groups had similar stadial period (6·71 days). The longest (8·89±1·47) and shortest third stadia (7·2±1·71) were observed among the offsprings of groups (iv) and (iii) females respectively. The duration of fourth stadium was similar to that of the first stadium.

Mating had influenced emergence of males from the fifth nymphal instars as evidenced by table 3. Both male and female offsprings of group (ii) emerged after 21·33±12·54 and 27·53±12·93 days respectively, while the groups (iii) and (iv) males and females emerged between 9·4±2·7 to 11±0 days.

The total nymphal period (I instar to adult) was significantly ($P=0{\cdot}05$) lower in the groups (iii) and (iv) than that of the group (ii). The present observation further indicated that the shortening of the total nymphal period in the groups (iii) and (iv) led the quicker emergence of adults of these categories.

Lowest nymphal mortality was observed among the offsprings of group (ii), though they had the longest stadial period. There was no significant difference in the nymphal mortality of offsprings of groups (iii) and (iv). From the present observations, no relationship could be established between the impact of mating and the nymphal mortality (table 4).

Mating had a positive, proportionate and significant ($P=0{\cdot}1$) influence on the longevity of males and females of *C. soosaii*. Male and female offsprings of group (iv) lived longer (23·5±1·29 and 37·0±4·24 days respectively). The shortest longevity was observed among the offsprings of group (ii) (19·28±1·5 and

Table 2. Impact of mating on incubation period and hatchability in *Coranus soosaii*, n = 6, ± SD.

Parameters	Categories of females		
	(ii)	(iii)	(iv)
Incubation period in days	7·64 ± 1·58	8·2 ± 0·84	8·6 ± 1·12
Number of nymphs hatched	19·25 ± 3·6	22·75* ± 4·2	3·8 ± 4·8
Hatching percentage	43·18 ± 7·12	49·05* ± 8·02	50·65* ± 9·6
Frequency of 0% hatching	6·0 ± 3·12	4·0* ± 3·46	3·67* ± 2·52
Frequency of 100% hatching	3·0 ± 2·32	4·75* ± 2·59	5·03* ± 2·08

*Significance shown at $P < 0{\cdot}1$.

Table 3. Impact of mating on stadial period in days in *Coranus soosaii*.

Categories of females	Stadial period						
	I to II	II to III	III to IV	IV to V	V to male	V to female	I to adult
(ii)	8·9 ± 1·92 (77)	6·71 ± 1·43 (58)	7·64 ± 1·8 (40)	7·5 ± 1·65 (29)	21·33 ± 12·54 (7)	27·53 ± 12·93 (4)	55·21 ± 19·53 (11)
(iii)	8·98 ± 2·14 (86)	6·71 ± 1·51 (55)	7·2 ± 1·71 (44)	8·0 ± 2·21 (31)	9·4 ± 2·7 (6)	12·0 ± 0·0 (3)	41·59 ± 8·92 (9)
(iv)	8·98 ± 2·04 (78)	6·46 ± 0·9 (50)	8·89 ± 1·47 (37)	8·38 ± 2·55 (28)	10·4 ± 0·89 (4)	11·0 ± 0·0 (3)	43·41 ± 7·4 (7)

Numbers in parentheses indicate the number of observations.

Table 4. Impact of mating on nymphal mortality (%) in *Coranus soosaii*.

Categories of females	Nymphal stages					
	I	II	III	IV	V	I to V
(ii)	16·88 (77)	20·69 (58)	12·5 (40)	17·24 (29)	16·5 (18)	49·35 (77)
(iii)	29·67 (86)	9·09 (65)	15·9 (44)	29·03 (31)	6·35 (16)	54·65 (86)
(iv)	28·20 (78)	14·0 (50)	8·10 (37)	17·86 (28)	23·53 (17)	52·56 (78)

Numbers in parentheses indicate the number of observations.

20·25 ± 1·5 days for males and females respectively). The male and female offsprings of group (iii) lived for 22·83 ± 3·54 and 24·66 ± 1·15 days respectively (table 5).

Offsprings emerging from all the 3 mated categories were male biased. But the highest female ratio was found in the offsprings of group (iv) (table 5). Further studies are needed to establish the impact of mating on the sex ratio of *C. soosaii*.

4. Discussion

Mating significantly influenced the length of preoviposition period in *C. soosaii*. Similar observations were already reported by Odhiambo (1968), Odhiambo and

Table 5. Impact of mating on adult longevity in days and sex ratio in *Coranus soosaii*, $\bar{X} \pm SD$.

Categories of females	Adult longevity		Sex ratio	
	Male	Female	Male	Female
(ii)	$19 \cdot 28 \pm 1 \cdot 5$ (7)	$20 \cdot 25 \pm 1 \cdot 5$ (4)	1·0 (7)	0·57 (4)
(iii)	$22 \cdot 83 \pm 3 \cdot 54$ (6)	$24 \cdot 66 \pm 1 \cdot 15$ (3)	1·0 (6)	0·5 (3)
(iv)	$23 \cdot 5 \pm 1 \cdot 29$ (4)	$37 \cdot 0 \pm 4 \cdot 24$ (3)	1·0 (4)	0·75 (3)

Numbers in parentheses indicate the number of observations.

Arora (1973), Ambrose and Livingstone (1979, 1985a, b, 1987) and Vennison and Ambrose (1986). Further, mating enhanced the rate of fecundity in this reduviid. Virgins laid comparatively less number of eggs with less amount of cementing material and the eggs shrank after a few days as reported by Lee (1954), Ryckman (1958), Odhiambo (1968), Ambrose and Livingstone (1979, 1985a, b, 1987) and Vennison and Ambrose (1986). Virgins also laid less number of batches of eggs. Total loss or less fecundity was also reported in *Haematosiphon inorodorus* (Lee 1954) and *Hesperocimex sonorensis* (Ryckman 1958). Similar observations were also recorded in other oriental reduviids, such as, *A. pedestris*, *A. siva*, *R. marginatus* and *R. kumarii* (Ambrose and Livingstone 1979, 1985a, b, 1987). Results showed that number of matings had a positive influence on the fecundity of *C. soosaii* unlike in *R. fuscipes* (Vennison and Ambrose 1986).

Mating had profound influence on hatchability too. Hatchability was directly proportional to the fecundity as reported for *A. pedestris*, *A. siva*, *R. marginatus* and *R. kumarii* (Ambrose and Livingstone 1979, 1985a, b, 1987). Thus mating had a positive proportionate impact on the hatchability. This might be due to the higher frequencies of 100% hatching and lower frequencies of 0% hatching. Therefore, it is also presumed that the possibility of receiving viable sperms was better ensured in (iv) group females. Under natural conditions these bugs were observed to migrate from one locality to another in search of prey which might provide with more opportunities of polyandry for the females. This facility might be enhanced by the availability of more number of younger males as their longevity ($23 \cdot 5 \pm 1 \cdot 29$) was considerably less than that of the females ($37 \pm 4 \cdot 24$). Thus polyandry serves as an effective measure for maintaining a steady level of natural population of this bug. This observation corroborates the earlier observations of Ambrose and Livingstone (1979, 1985a, b, 1987) and differs from that of Vennison and Ambrose (1986). The data further indicated that the females parental ageing caused non-viability of eggs. Similar observation was also reported by Rabinovich (1972).

The highest hatchability recorded in the (iv) group females might be due to the least frequency of 0% hatching ($3 \cdot 67 \pm 2 \cdot 08$) recorded in this group. In the groups (ii) and (iii) too, a direct correlation could be established between the frequencies of 0% and 100% hatching and the hatchability as observed in the females mated with different males of different ages. This suggests that the higher fertility observed in the females of groups (iii) and (iv) might be due to the availability of more number

of viable sperms as reported by Rabinovich (1972) and Ambrose and Livingstone (1979, 1985a, b).

The present observations show that mating had no positive impact on moulting but on emergence. Though mating did not have significant influence on nymphal mortality and sex ratio it certainly extended the longevity of *C. soosaii*. In summary, mating shortened pre-oviposition period, enhanced both fecundity and hatchability, hastened the emergence and extended adult longevity.

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Effect of *Azadirachta indica* and *Pongamia glabra* leaf extracts on food utilization and modulation of efficiency of digestive enzymes in *Euproctis fraterna* (Lepidoptera: Lymantridae)

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Abstract. The effect of application of *Azadirachta indica* and *Pongamia glabra* leaf extracts on food consumption and utilization in fifth instar larvae of *Euproctis fraterna*, a serious lepidopterous pest on castor, *Ricinus communis*, is assessed. Reduction in food consumption by *Euproctis fraterna* feeding on castor leaves treated with extract of *Azadirachta indica* was 11% higher than that recorded for castor leaves treated with *Pongamia glabra*. Consumption, assimilation, production and their rates showed a negative correlation with the concentration of the extracts used.

The effect of administration of these two leaf extracts on efficiency of the digestive enzymes invertase, amylase and protease of *Euproctis fraterna* was also assessed. A reduction in activity of these enzymes with the administration of increasing concentration of extracts was also observed.

Leaf extracts of neem and *Pongamia* appear to be most efficaceous in controlling *Euproctis fraterna* especially when they are administrated along with castor leaves in the fifth instar providing a suitable alternative to synthetic pesticides.

Keywords. Leaf extract; *Azadirachta indica*; *Pongamia glabra*; food consumption; food utilization; enzyme activity; *Euproctis fraterna*; *Ricinus communis*.

1. Introduction

It is being increasingly realised that the potential of several secondary chemical compounds in plants to disrupt or interrupt specific mechanism involving metamorphosis, nutrition, reproduction and behaviour of insects could be exploited in the control of insect pest, offering a safer alternative to the conventional pesticide use (Ananthakrishnan 1987). In this context, use of extracts of such plants like *Azadirachta indica* is gaining momentum since azadirachtin (Aza) is a feeding inhibitor and growth disrupting compound for most insect orders (Garcia *et al* 1986). It affects growth and development (Leuschner 1972; Meisner *et al* 1976, 1978), reduces oviposition (Jacobson *et al* 1978) or interferes with the insect's endocrine system (Sieber and Rembold 1983). Whereas some investigations have been carried out on the efficacy of extracts in seeds and roots of *Azadirachta* and *Pongamia* respectively (Butterworth and Morgan 1971; Srimannarayana *et al* 1987) on the control of insect pests. There is paucity of information on the role of leaf extracts of these plants as an effective antifeedant (Hussain and Masood 1975; Eganjobi and Afolami 1976; Rossner and Zenbity 1986). Hence the present study aims at assessing the effect of application of *A. indica* and *P. glabra* leaf extracts on the food consumption and utilization in *Euproctis fraterna*, a serious lepidopterous pest on castor, *Ricinus communis*. Consumption, digestion and utilization of food plants by herbivorous insect pests are important since consumption indices are

considered to be indirect measurement of the relative susceptibilities of different varieties of crops to pest infestation (Dandapani and Balasubramanian 1980).

Digestive enzymes in insects are generally adapted to the diet on which the species feed (Wigglesworth 1965). Nutritional and environmental factors affect digestive enzymes (Waldbauer 1962; Soo Hoo and Fraenkel 1966; Nalinasundari *et al* 1987). In some cases digestive enzymes can be used as parameters for assessing antifeeding activity (Ishaaya *et al* 1974, 1977, 1980, 1982). Despite availability of ample information concerning biochemical properties of digestive enzymes in various insects (House 1974; Wigglesworth 1974), relatively little is known about their role in insect feeding and insect-host compatibility. Hence, an attempt has been made to assess the effect of administration of *A. indica* and *P. glabra* leaf extracts on efficiency of digestive enzymes invertase, amylase and protease of *E. fraterna*.

2. Materials and methods

The larvae of *E. fraterna* were collected from Thiagarajar College campus and were reared in the laboratory on the leaves of castor (LD 12:12; temp. 30 ± 1°C at 80% RH). Acetone extract of *A. indica* and *P. glabra* leaves (25 g) were taken in soxhlett for 8 h at 65 ± 2°C and were evaporated to dryness (taken as 100% concentration). Different concentrations (ppm) were prepared from the above extract by weighing 25 mg of neem and *Pongamia* leaf extracts and dissolving it in 2 ml of acetone and 98 ml of distilled water was added to this to make 100 ml. This gives 250 ppm concentration. Likewise 500, 750 and 1000 ppm were prepared by weighing 50, 75, 100 mg of both extracts and dissolving it in the same procedure.

Third instar larvae of *E. fraterna* were selected and fed with castor leaves soaked in different concentrations of extracts and ED_{50} values were assessed. Growth inhibition at different concentrations were plotted and ED_{50} values were found for the two extracts (figure 1 and table 1).

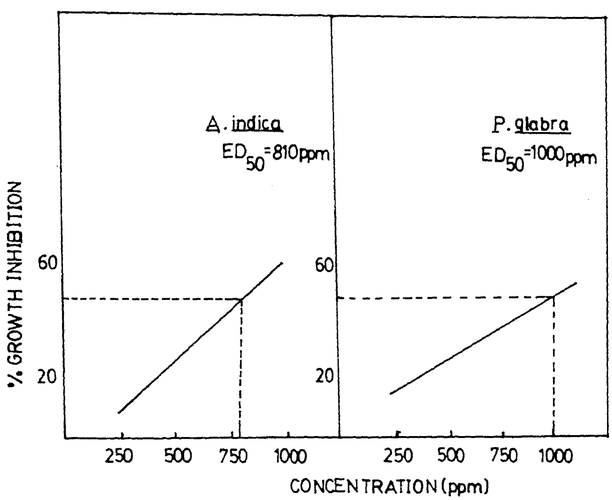

Figure 1. ED_{50} value of third instar larvae of *E. fraterna* treated with different doses of *A. indica* and *P. glabra* leaf extracts.

Table 1. ED_{50} value for third instar larva of *E. fraterna.*

Plant extract used	ED_{50}* value (ppm)
A. indica	810
P. glabra	1000

*Dose required to produce 50% inhibition in growth.

For oral administration experiment, fifth instar larvae were allowed to feed on treated castor leaves and in control experiments, larvae were fed on untreated castor leaves. The effect of plant extracts was assessed on conversion and utilization efficiencies of this pest fed on castor leaves.

2.1 *Calculation of feeding budget*

The scheme of feeding budget followed in the present work is that of the IBP formula (Petrusewicz and Macfadyen 1970) usually represented as $C=F+U+R+P$, where C is the food energy consumed, F the energy of faeces egested, U the nitrogenous waste excreted, R the energy spent on metabolism and P the growth. Rates of feeding, conversion and metabolism as well as assimilation efficiency and conversion efficiency of the larva were calculated by the following formulae:

$$\text{Conversion rate } (Cr) = \frac{C \text{ (mg/individual)}}{\text{Mid body wt. (g)} \times \text{duration (day)}}.$$

$$\text{Assimilation rate } (Ar) = \frac{A \text{ (mg/individual)}}{\text{Mid body wt. (g)} \times \text{duration (day)}}.$$

$$\text{Production rate } (Pr) = \frac{C \text{ (mg/individual)}}{\text{Mid body wt. (g)} \times \text{duration (day)}}.$$

$$\text{Assimilation efficiency } (AD) = A/C \times 100.$$

$$\text{Gross production efficiency } (ECI) = P/C \times 100.$$

$$\text{Net production efficiency } (ECD) = P/A \times 100.$$

2.2 *Enzyme bioassay*

2.2a *Preparation of larval enzyme solution and enzyme assays*: Extracts of *A. indica* and *P. glabra*-treated castor leaf-fed fifth instar larvae were anaesthetized with cotton pads soaked in chloroform. The gut of each fifth instar larva was dissected free from other tissues by cutting off the larval head with a razor blade and removing the alimentary canal on a wet filter paper using a fine forceps assisted by a slight pressure at the posterior end of the body. The gut was homogenized for 3 min at 3°C in ice-cold citrate-phosphate buffer (pH 6·8) using a chilled tissue

grinder. Brei of the gut was suspended in ice-cold buffer and made up to 1 ml. The homogenate was spun at 12,000 g for 15 min. The supernatant was used as enzyme source. Amylase and invertase activities were determined adopting the method of Ishaaya and Swirsky (1970) and protease by the method of Birk *et al* (1962).

3. Results

3.1 *Consumption and utilization*

The quantity of food consumed by *E. fraterna* was found to be lower when fed on castor leaves treated with leaf extracts of *A. indica* and *P. glabra.* Reduction in food consumption by *E. fraterna* feeding on castor leaves treated with extract of *A. indica* was 11% higher than that recorded for castor leaves treated with *P. glabra* (table 2). This variation is highly significant as shown by *t*-test value (table 2). The concentration of extracts and consumption are significantly correlated negatively (figure 2). Consumption rate (*Cr*) is an index of damage inflicted by insect pests on plants. There was a significant reduction in the rate of food consumption in the later stages of development when compared to control reflecting the level of damage to the tune of 84·325 mg/g/body wt./day in *A. indica* extract-treated insect and 97·843 mg/g/body wt./day in *P. glabra*-treated insect. Whereas the value for the control experiment was as high as 120·338 mg/g/body wt./day (table 2). Two-way analysis of variance reveal that concentration of the extracts and age of the larva significantly alter the consumption rate of the pest ($F=1236$, $P<0{\cdot}001$).

3.2 *Food conversion*

A reduction in the quantity of food converted into body weight was observed in *E. fraterna* fed on castor leaves treated with leaf extracts of *A. indica* and *P. glabra.* Percentage of reduction in food converted by *E. fraterna* feeding on castor leaves treated with leaf extracts of *A. indica* was higher (22·66) than that recorded for the food treated with *P. glabra* (17·13) (table 2). This variation is highly significant as shown by '*t*' test value. Apparently there is a significant reduction in the rate of conversion (10·206 mg/g/body wt./day fed on *A. indica*-treated castor leaf and 10·166 mg/g/body wt./day fed on *P. glabra*-treated castor leaf) when compared to control (12·152 mg/g/body wt./day). Two-way analysis of variance reveals that concentration of the extract and age of the larva significantly alter the conversion rate ($F=30{\cdot}02$, $P<0{\cdot}001$ with reference to *A. indica*-treated food and $F=179{\cdot}17$, $P<0{\cdot}001$ with reference to *P. glabra*-treated food).

3.3 *Assimilation and growth efficiencies*

Assimilation efficiency (*AD*) showed a decline when insects were fed on leaves treated with extracts of *A. indica* and *P. glabra.* Percentage of decline in assimilation efficiency compared with control was around 15 when larvae were fed on a maximum quantity of 1000 ppm extract (table 2). Two-way ANOVA for both the extracts reveals that the approximate digestibility was significantly influenced by the concentration of the extract and age of the larva ($F=1467$, $P<0{\cdot}001$ with reference

Table 2. Effect of *A. indica* and *P. glabra* leaf extracts on the feeding parameters of fifth instar larva of *E. fraterna*.

Concentration (ppm)	*C*	*A*	*P*	*M*	*Cr*	*Ar*	*Pr*	*Mr*	*AD*	*ECI*	*ECD*
A. indica											
Control	46·517 ± 0·490	36·000 ± 0·560	4·355 ± 0·449	31·645 ± 0·792	120·338 ± 0·439	94·04 ± 0·154	12·152 ± 0·168	81·268 ± 0·295	78·068 ± 0·130	10·545 ± 0·456	13·248 ± 0·463
250	42·537 ± 0·487	30·255 ± 0·595	4·482 ± 0·422	25·773 ± 0·587	108·500 ± 0·488	77·00 ± 0·575	12·285 ± 0·306	65·183 ± 0·374	71·35 ± 0·685	11·445 ± 0·358	15·200 ± 0·434
500	38·172 ± 0·452	27·352 ± 0·514	4·155 ± 0·324	22·000 ± 0·778	99·168 ± 0·363	71·00 ± 0·475	12·355 ± 0·288	59·47 ± 0·416	72·498 ± 0·451	12·100 ± 0·193	17·200 ± 0·428
750	33·115 ± 0·383	23·000 ± 0·565	4·227 ± 0·288	18·773 ± 0·224	86·380 ± 0·416	60·32 ± 0·434	12·183 ± 0·133	48·052 ± 0·862	69·488 ± 0·458	14·278 ± 0·358	20·300 ± 0·598
1000	31·05 ± 0·485	19·600 ± 0·527	3·368 ± 0·414	16·292 ± 0·453	84·323 ± 0·425	53·54 ± 0·382	10·206 ± 0·360	43·077 ± 0·628	63·405 ± 0·507	10·847 ± 0·503	17·131 ± 0·395
	$t = 47·52$	$t = 47·52$	$t = 31·61$								$t = 5·727$
	$P < 0·001$	$P < 0·001$	$P < 0·01$								$P < 0·001$
P. glabra											
Control	46·517 ± 0·490	36·000 ± 0·560	4·355 ± 0·449	31·645 ± 0·792	120·338 ± 0·439	94·04 ± 0·154	12·152 ± 0·168	81·268 ± 0·295	78·068 ± 0·130	10·545 ± 0·456	13·248 ± 0·463
250	46·100 ± 0·260	34·200 ± 0·159	4·125 ± 0·554	30·075 ± 0·654	119·00 ± 0·741	87·000 ± 1·000	12·323 ± 0·489	75·000 ± 0·453	72·383 ± 0·962	10·195 ± 0·640	14·186 ± 0·680
500	45·053 ± 0·131	29·163 ± 0·325	4·268 ± 0·350	24·895 ± 0·625	118·000 ± 0·602	77·347 ± 0·736	12·387 ± 0·481	65·113 ± 0·725	65·255 ± 0·725	10·140 ± 0·366	16·314 ± 0·760
750	41·420 ± 0·329	27·220 ± 0·257	4·150 ± 0·313	23·070 ± 2·348	110·000 ± 0·921	73·294 ± 0·470	11·658 ± 0·936	60·277 ± 0·727	66·069 ± 0·784	11·082 ± 0·648	16·865 ± 0·563
1000	36·113 ± 0·515	23·118 ± 0·260	3·609 ± 0·345	19·509 ± 1·251	97·843 ± 0·987	63·000 ± 0·732	10·166 ± 0·700	85·000 ± 0·956	64·115 ± 0·619	9·993 ± 0·667	15·611 ± 1·036
	$t = 40·79$	$t = 46·67$	$t = 3·00$								$t = 3·744$
	$P < 0·001$	$P < 0·001$	$P < 0·01$								$P < 0·01$

Mean ± SD represents an average of 5 replicates.

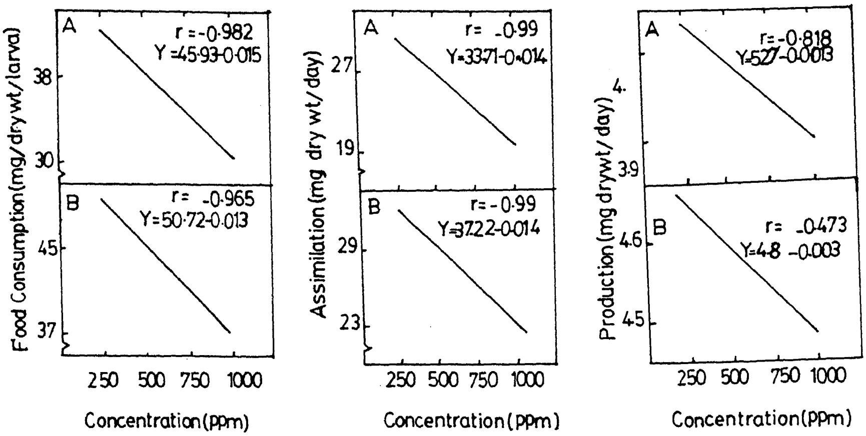

Figure 2. Relationship between food consumption, assimilation and production of fifth instar larva of *E. fraterna* and concentration of the leaf extracts of *A. indica* (A) and *P. glabra* (B).

to *A. indica*-treated food and $F = 103$, $P < 0{\cdot}001$ with reference to *P. glabra*-treated food). However, growth efficiency of the pest was not affected by these two treatments with extracts whereas this pest developed a compensatory mechanism by increasing its growth efficiency which is statistically significant.

3.4 *Impact on enzyme activity*

Digestive enzymes can be used as parameters for assessing antifeedant activity. Treatment of fifth instar larvae of *E. fraterna* with extracts of *A. indica* and *P. glabra* along with castor food showed a decreased activity of digestive enzymes invertase, amylase and protease (table 3). A greater inhibition was found in larvae consuming *A. indica* extract-treated food than in *P. glabra* extract-treated food. This variation is highly significant as evidenced by '*t*' test value. The concentration of the extracts and activity of invertase, amylase and protease are significantly correlated negatively. Two way ANOVA for both the extracts reveals that the activity of the enzymes invertase, amylase and protease was significantly influenced by the concentration of the extracts and age of the larvae. The following *F* values were calculated:

For invertase

$F = 5{\cdot}26$ $P < 0{\cdot}01$ (*A. indica*-treated food).
$F = 3{\cdot}12$ $P < 0{\cdot}01$ (*P. glabra*-treated food).

For amylase

$F = 5{\cdot}50$ $P < 0{\cdot}01$ (*A. indica*-treated food).
$F = 3{\cdot}48$ $P < 0{\cdot}01$ (*P. glabra*-treated food).

For protease

$F = 12{\cdot}22$ $P < 0{\cdot}001$ (*A. indica*-treated food).
$F = 30{\cdot}74$ $P < 0{\cdot}001$ (*P. glabra*-treated food).

Table 3. Effect of *A. indica* and *P. glabra* leaf extracts on the enzyme activity of invertase μg glucose/reaction, amylase μg maltose/reaction and protease μg tyrosine/reaction in fifth instar larva of *E. fraterna.*

Concentration (ppm)	Invertase μg/glucose activity	Amylase μg/maltose activity	Protease μg/tyrosine activity
A. indica			
Control	2000·00 ± 319·94	5090·90 ± 562·45	362·50 ± 17·62
250	1520·27 ± 37·56	4064·50 ± 83·00	322·22 ± 17·11
500	1077·92 ± 25·62	3553·84 ± 242·64	289·99 ± 63·00
750	988·09 ± 17·03	3043·47 ± 52·50	236·36 ± 3·28
1000	874·41*	2000·00*	205·26*
	$t = 143{\cdot}706$ $P < 0{\cdot}001$	$t = 272{\cdot}78$ $P < 0{\cdot}001$	$t = 8{\cdot}365$ $P < 0{\cdot}001$
P. glabra			
Control	2000·00 ± 319·94	5090·90 ± 562·45	362·50 ± 17·62
250	1092·60 ± 27·09	4554·16 ± 48·86	348·00 ± 9·80
500	1064·10 ± 26·00	4000·00 ± 295·32	322·22 ± 15·81
750	1000·00 ± 67·08	3000·00 ± 269·80	251·61 ± 5·75
1000	943·18* ± 84·38	2876·74* ± 23·50	216·66* ± 7·81
	$t = 147{\cdot}97$ $P < 0{\cdot}001$	$t = 251{\cdot}75$ $P < 0{\cdot}001$	$t = 8{\cdot}618$ $P < 0{\cdot}001$

*Control vs 1000 ppm.
Mean ± SD represents average performance of 5 individuals.

A direct significant correlation exists between the amount of food consumed and the activity of the 3 enzymes invertase, amylase and protease ($P = < 0{\cdot}001$) (figure 3).

3.5 *Morphological deformities*

Varying degrees of deformities such as 'larval-pupal' intermediates were produced when treated with 1000 ppm of neem and *Pongamia* extracts. The emerging adult was quite deformed.

4. Discussion

Neem extracts contain azadirachtin which is tetranortriterpenoid (Garcia *et al* 1984). This azadirachtin from *A. indica* as well as karanjin, pongapin, 3-methoxy pongapin and globachrome from *P. glabra* in general inhibit the physiological functions of the insects and have pronounced antifeedant effect (Butterworth and Morgan 1971; Zanno *et al* 1975; Parmar *et al* 1976; Rembold *et al* 1982; Steffens and Schmutterer 1982; Dorn *et al* 1986; Srimannarayana *et al* 1987). In the present study *E. fraterna* consumed less quantity of food when treated with *A. indica* and *P. glabra* extracts and this reduction in consumption led to diminished feeding rate in both cases. Earlier reports in support of the present findings indicate that in *E. fraterna* the extracts of *Datura metal* caused greater reduction in food consumption and in its feeding rates (Chockalingam *et al* 1983; Nalinasundari 1988). This reduced food consumption might be explained by a behavioural antifeedant effect due to perception by the insects peripheral chemoreceptors

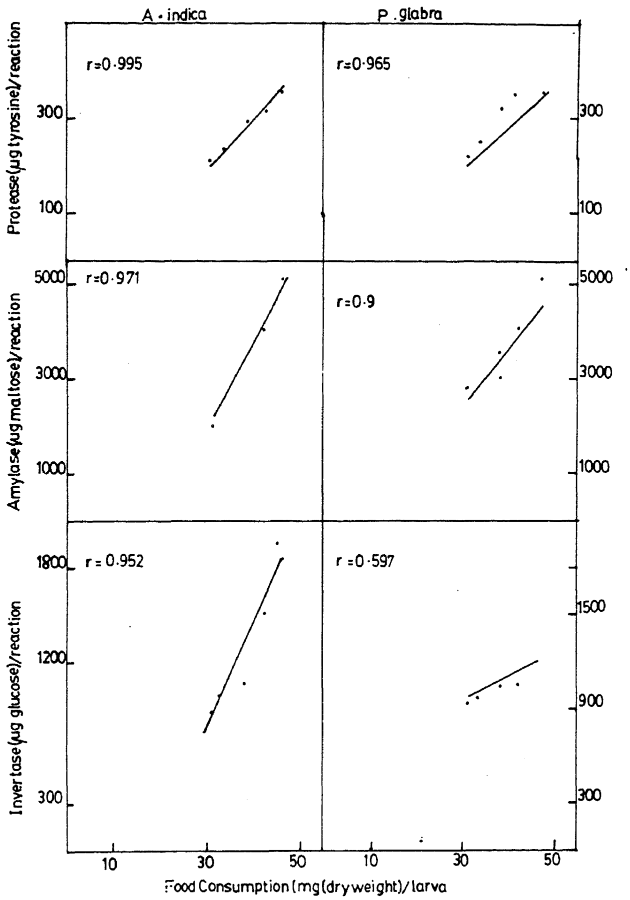

Figure 3. Relationship between food consumption and activity of digestive enzymes treated with extracts of *A. indica* and *P. glabra* to fifth instar larva of *E. fraterna*.

(Bernays 1981). Consumption, assimilation, production and their rates showed a negative correlation with the concentration of the extracts used. This is in conformity with the findings on the use of *Eucalyptus globulus* on food utilization of *E. fraterna* (Nalinasundari 1988). Plant products possess antiherbivore chemicals like tannins, phenol (Hillies 1966) and essential oils (Penfold and Willis 1961) which adversely affected the growth of the insect and its metabolic efficiency (Feeny 1970; Levin 1971; Morrow and Fox 1980).

The polyphagous insect, *E. fraterna* was unable to gain a significant increase in weight compared to control when this insect was subjected to the toxic stress of the extracts of *A. indica* and *P. glabra* leaves (table 2). Studies on the effect of neem oil extractive (NOE) on the food utilization efficiency in *Spodoptera litura* led to the conclusion of the fact that the azadirachtin compound of the NOE might have inhibited proper growth and growth rate (Chockalingam *et al* 1983). This fact has also been substantiated by the present study. Dryer *et al* (1979) showed that pinitol, a fatty acid extract from soya bean leaves reduced the weight gained by the larvae of *Heliothis zea*. Since NOE contains 53% of fatty acid (Attri and Prasad 1980) it is

likely that this higher concentration of fatty acid might have been responsible in blocking the pores of the cellular membrane of the alimentary canal of *E. fraterna* fed on neem extract-treated castor leaf resulting in reduction in growth and growth rates. Deterrancy is also reflected by decreased weight gain (Fagoone 1983). Meisner *et al* (1981) also reported small weight gains in *Spodoptera littoralis* larvae with fairly high concentration (0·002% and above) of azadirachtin.

Reduction in assimilation efficiency of *E. fraterna* fed on castor leaves treated with neem and *Pongamia* extracts might be due to reduced activities of digestive enzymes. The hypothesis put forward by Singleton and Kratzer (1973) implicates direct inhibition of digestive enzymes by the extracts. Growth efficiency of the insect appears to have been enhanced by increasing the dosage of both the plant extracts (table 2). A similar pattern of enhanced growth efficiency was also reported in the larvae of *Crocidolomia linotalis* suggesting the fact that this insect is capable of detoxifying to some extent (Fagoone 1983). This explanation may hold good with regard to the enhanced growth efficiency noticed in the present study on *E. fraterna.*

Gut invertase, amylase and protease activities in fifth instar larvae of *E. fraterna* fed on *A. indica* and *P. glabra*-treated castor leaves were found to be inhibited. Ishaaya and Casida (1975) reported that inhibition of digestive enzymes of *Tribolium castaneum* larvae by phenyltin compound which are antifeedants. They also confirmed the fact that inhibition of protease activity due to the antifeedant compound may be a direct probably acting on a physiological system affecting protease activity. Direct inhibition of the 3 digestive enzymes due to the impact of antifeedant compounds azadirachtin found in *A. indica* and Karanjin, 3-methoxy pongapin and globachrome found in *P. glabra* noted in the present investigation is in conformity with the observations of Ishaaya *et al* (1974) and Ishaaya and Casida (1975).

A direct correlation was obtained between decreased food consumption and the activity of invertase, amylase, protease in the present investigation. This lowered food consumption may be due to the presence of antifeedant compound in the diet which in turn inhibits digestive enzymes. Ishaaya and Casida (1975) also suggested that retardation in larval growth may result from a lower feeding level caused by the reduced activity of the larval digestive enzymes.

The results of the present investigation reinforce the earlier findings that extracts of neem and *Pongamia* appear to be most efficaceous in the control of *E. fraterna* especially when they are administered along with castor leaves in the fifth instar thereby paving the way for effective pest management and providing suitable alternative to synthetic pesticides.

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Insect growth regulator XRD-473 (OMS 3031), a prospective compound for control of mosquito vectors

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Abstract. Insect growth regulating activity of a substituted urea compound XRD-473 (OMS 3031) was evaluated against the target species viz. *Culex quinquefasciatus*, *Aedes aegypti*, *Anopheles stephensi* and non target species *Toxorhynchites splendens*. This compound inhibited the emergence of all these mosquito species with EI_{50} values of 9×10^{-5}, $1{\cdot}09 \times 10^{-4}$, $2{\cdot}22 \times 10^{-4}$ and $2{\cdot}14 \times 10^{-4}$ mg (ai)/l respectively. Emergence inhibiting activity of XRD-473 was found to be more than fenoxycarb, and S 21149 against one or other species. In stagnant polluted water, the activity of the compound against *Culex quinquefasciatus* was for shorter duration of 5 and 10 days at 0·02 and 0·2 kg(ai)/ha respectively whereas in clear water the activity was for longer duration i.e. 11 and 17 days at the same dosage. However, in drain the activity was negligible i.e. 12 days at 2 kg(ai)/ha. More than two weeks control of *Aedes aegypti* was obtained in cement tank at the treatment rate of 0·2 kg(ai)/ha whereas at the lower dose of 0·02 kg(ai)/ha this compound was effective for less than a week.

Keywords. Insect growth regulators; emergence inhibition: *Culex quinquefasciatus*: *Anopheles stephensi*: *Aedes aegypti*: XRD-473.

1. Introduction

Insect growth regulators or the third generation insecticides (Williams 1967) have emerged as a new frontier for insect control and have developed as a result of rational leads from basic entomological research on metabolic disruptors, moult inhibitors and behaviour modifiers of insects. Since the target site of action for these chemicals is specific and are known to disrupt only in certain species at certain times during the life cycle, these materials are thought to have fewer serious deleterious effects on the environment and also on non-target organisms (Retnakaran *et al* 1985). Though a large number of chemicals of this category were synthesized and evaluated, a few such as Dimilin (Ten Housten *et al* 1980; Ho *et al* 1987), methoprene (Dame *et al* 1976), penfluron and furyltriazine (Sexana and Kaushik 1986), fenoxycarb (Mulla *et al* 1985; Tyagi *et al* 1987), S 21149 (an oxime) and S 31183 (pyridine) (Estrada and Mulla 1986; Amalraj *et al* 1988a, b), OMS 3009, OMS 3013 and OMS 2015 (Amalraj *et al* 1988a, b) etc have been tried successfully against mosquito vectors. This paper highlights the effect of a benzoyl urea compound, XRD-473 (OMS 3031) against major vector mosquito species.

2. Materials and methods

XRD-473 (OMS-3031), a substituted urea compound N-[(((3, 5-dichloro-4-(1, 1, 2, 2, tetrafluoroethoxy)-phenyl)amino)carbonyl)-2, 6-difluorobenzamide], was obtained from Dow Chemical International Ltd, USA through WHO as 5% emulsifiable

concentrate (EC) for evaluating its insect growth regulating activity against vector mosquitoes.

2.1 *Laboratory evaluation*

The 5% EC formulation of XRD-473 was first dissolved in acetone and then suitably diluted for the required concentrations. In order to obtain different target doses, 1 ml of stock solution of appropriate concentration was added in 499 ml of water in enamel trays (15 × 10 × 5 cm). To each tray, 50 third instar larvae of test species were added. Four replicates were set for each concentration per test and a control was maintained for each species. The test was carried out at room temperature 29 ± 1°C and humidity 60–70%. The larvae were provided with larval food. Mortality in each stage i.e. 3rd, 4th and pupae and the number of emerged adults from live pupae were recorded. Total emergence inhibition was calculated taking into account the mortality in all the stages from 3rd stage larvae to pupae including partly emerged adults and adults with morphological deformities. Emergence inhibition (EI_{50} and EI_{90}) was estimated by regression analysis (WHO 1975).

Pupae of all the mosquito species were also exposed at different concentrations (i.e. 0·01, 0·1 and 1 mg/l) and emergence inhibition was determined. Dead larvae, pupae and larval-pupal intermediates and partly enclosed adults were observed for morphological abnormalities.

2.2 *Field evaluation*

A preliminary survey of all the breeding habitats of *Culex quinquefasciatus* and *Aedes aegypti* in and around the study area was done and those habitats which supported heavy mosquito breeding were taken for trial. XRD-473, was evaluated against *Cx. quinquefasciatus* in different breeding habitats at varying rates (i.e. 0·02, 0·2 and 2 kg(ai)/ha). Four replicates were maintained for each concentration and a separate control was kept for each type of habitat. The compound was sprayed with the help of a hand sprayer, with a discharge rate of about 160 ml/min.

Density of immatures and rate of adult emergence were monitored before and subsequently on alternative days after treatment. Four dips at periphery and one at the centre per treated habitat were pooled in a tray and stage wise counting was done. Known number of live pupae and fourth instar larvae were brought to the laboratory for observing adult emergence. In cases where the treated habitat was negative of all stages, water samples were brought to the laboratory and known number of laboratory reared third instar larvae were added and observed for the emergence inhibition. Emergence inhibition (% EI) was calculated by the following formula:

$$\text{Emergence inhibition (\%)} = 100 - \frac{\text{(No. of adults emerged)}}{\text{(No. of pupae collected)}} \times 100.$$

Effective duration in days was determined by noting down the days up to which more than 80% inhibition in adult emergence was noticed. Symptoms of disruption of moulting process typical of any insect growth regulator (IGR) compounds were observed and recorded.

3. Results

Emergence inhibition activity (EI_{50} and EI_{90}) of XRD-473 on all the 3 vector mosquito species and on the non-target mosquito *Toxorhynchites splendens* are presented in table 1. This compound was found to be very effective on *Cx. quinquefasciatus* and among the 3 vectors it was least effective on *Anopheles stephensi.*

Table 2 shows the effect of this compound (in terms of percentage inhibition) on the pupae of these mosquitoes. Just as in the case of the larvae, this compound was most effective on the pupae of *Cx. quinquefasciatus*, while least inhibition was seen within the pupae of *An. stephensi.* However, the pupae of *T. splendens* (non-target species) was also observed to have a high percentage of inhibition especially at 1 mg/l.

XRD-473 was evaluated against *Cx. quinquefasciatus* in different breeding habitats such as cesspits, cement tanks and drains at 3 different treatment rates, i.e. 0·02, 0·2 and 2 kg(ai)/ha and the results are presented in figure 1. In cesspits this compound was found to be effective (80% EI) for 11 days at 0·2 kg(ai)/ha. When tested at a lower concentration of 0·02 kg(ai)/ha it was effective only for 6 days. More than 80% EI was observed for nearly 26 days at high treatment rate of 2 kg(ai)/ha. In contrast, in cement tank relatively at lower rate (0·02 kg(ai)/ha), 100% EI was observed for 12 days, while at 0·2 kg(ai)/ha it was effective for 17 days. Since there was steady flow of water in drains the effectiveness of the compound was drastically reduced due to dilution and at 0·2 kg(ai)/ha this IGR was effective only for 4 days and at 2 kg(ai)/ha it was effective for 12 days.

The compound was effective for two weeks (17 days) against *Ae. aegypti* in cement tanks at the application rate of 0·2 kg(ai)/ha. Whereas at the lower rate of 0·02 kg(ai)/ha this compound was effective for less than a week (figure 1).

Heavy larval mortality, malformation of the pupae resulting either in immediate death or delayed mortality due to incomplete emergence of adults from the pupal cuticle was observed during the period of effectiveness.

Table 1. Laboratory evaluation of IGR OMS-3031 against 3rd instar larvae of mosquito species.

Species	Regression equation	EI_{50} (mg/l)	EI_{90} (mg/l)
Cx. quinquefasciatus	$Y = 8{\cdot}51 + 0{\cdot}38 \log X$	$9{\cdot}000482 \times 10^{-5}$	$2{\cdot}688419 \times 10^{-3}$
Ae. aegypti	$Y = 8{\cdot}50 + 0{\cdot}38 \log X$	$1{\cdot}099641 \times 10^{-4}$	$3{\cdot}059457 \times 10^{-3}$
An. stephensi	$Y = 10{\cdot}6090 + 0{\cdot}667 \log X$	$2{\cdot}226043 \times 10^{-4}$	$1{\cdot}516718 \times 10^{-3}$
T. splendens	$Y = 19{\cdot}7473 + 1{\cdot}745 \log X$	$2{\cdot}140856 \times 10^{-4}$	$4{\cdot}457339 \times 10^{-4}$

Table 2. Effect of IGR OMS-3031 on pupae of mosquito species in the laboratory.

	% EI at different dosages (mg/l)		
Species	0·01	0·1	1·0
Cx. quinquefasciatus	58	75	94
Ae. aegypti	34	70	82
An. stephensi	14	18	45
T. splendens	11	54	96

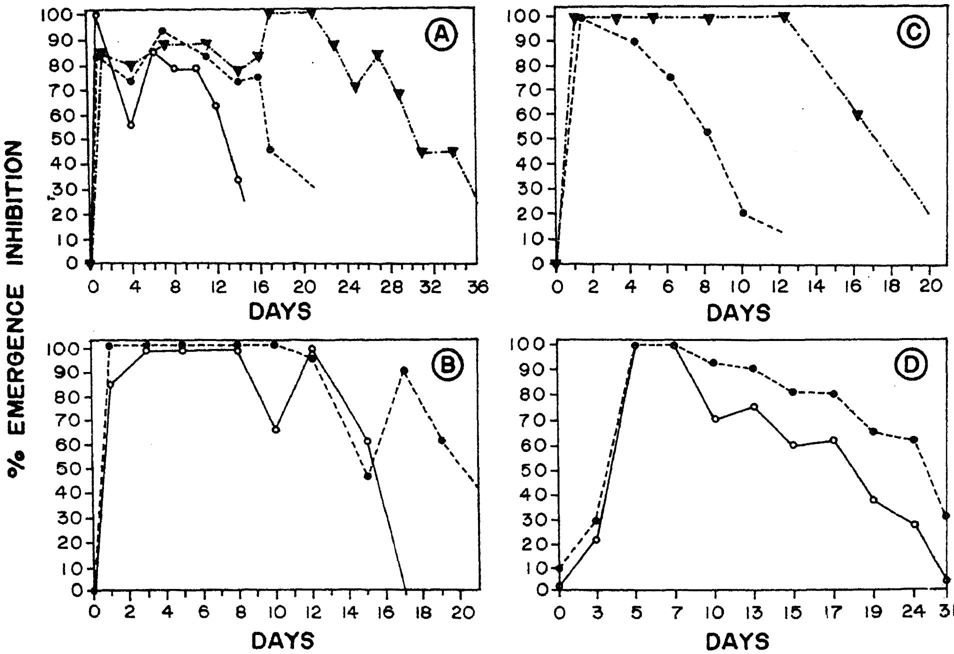

Figure 1. Field evaluation of XRD-473 (OMS 3031) against (i) *Culex quinquefasciatus* in (**A**) cesspits, (**B**) cement tanks and (**C**) drains and (ii) *Aedes aegypti* in (**D**) cement tanks. The dosages are 0·02 (○), 0·2 (●) and 2·0 (▼) kg(ai)/ha.

4. Discussion

The emergence inhibition activity of XRD-473 in vector mosquitoes in the laboratory was compared with that of other IGRs. In earlier studies (Tyagi *et al* 1987), fenoxycarb was found to induce 50% inhibition of adult emergence in *Cx. quinquefasciatus* at the concentration of 0·0017 mg/l which is 18 times more than the concentration required to get the same result with XRD-473. Similarly, when the results of S-21149, OMS 3009, OMS 3013 and OMS 2015 obtained under similar conditions (Amalraj *et al* 1988a, b) were compared with results obtained with XRD-473, it was found that the later compound was 154 times more effective than S-21149 against *Cx. quinquefasciatus* and as effective as S-21149 against *Ae. aegypti* and *An. stephensi.* EI_{50} of this compound and that of OMS 3009, OMS 3013 and OMS 2015 proved that all these IGRs are equally effective. This compound was less effective on the pupae which is in agreement with earlier observations with other IGRs.

The present study clearly shows the potential of this compound to inhibit emergence of *Cx. quinquefasciatus* in small confined breeding habitats such as cesspits at a dosage of 2 kg(ai)/ha for 26 days. Mulla and Darwazeh (1988) had reported that this compound was effective for 7 days at 0·056 kg(ai)/ha, in large dairy wastewater lagoons. In clear water bodies like cement tanks, the compound was effective for 17 days at 0·2 kg(ai)/ha and hence it would have to be applied fortnightly to check the breeding of these mosquitoes. Similarly in drains, this compound would have to be applied once in two weeks at 2 kg(ai)/ha to check the

proliferation of *Cx. quinquefasciatus*, a lower dosage may be sufficient in the case of stagnant blocked kutcha drains.

Though the effectiveness of this compound is limited to freshwater habitats, it can be incorporated in Integrated Vector Management programme. There are always some situations like profuse breeding of vector mosquitoes in cement tank, discarded tyres and drums in curing yards where water is clean but fish cannot be used either due to volume of water or due to unwillingness of people. In such situations IGRs can play useful role. Nonetheless, viewing the lethal effect of this compound on *T. splendens* there is imperative necessity for further study of this compound on non target organisms before being used in IVM programme.

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Field life-tables and key mortality factors of *Achaea janata* Linn on castor*

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Abstract. Field life-tables and key mortality factors of castor semilooper *Achaea janata* L. were studied on castor for the year 1985–86 and 1986–87. There were 4 regular overlapping generations during both the years. The parasitization of *Achaea janata* larvae in early instars (I–III instar) by parasite *Microplitis maculipennis* (Sziepligetis) was 1·34, 4·41, 22·02 and 43·70% in first, second, third and fourth generation respectively during 1985–86. Similarly, the parasitization by same parasite was 44·12, 61·46 and 65·55% during second, third and fourth generation respectively in the year 1986–87. Key mortality factors and separate budget for each generations were prepared.

Keywords. Field life-tables; key mortality factors; *Achaea janata* L.

1. Introduction

Field life-tables and key mortality factors may be analysed to determine what stage in the life cycle contributes the most to the population trend when series of life-tables are available (Deevey 1947; Harcourt 1963, 1969; Atwal and Bains 1974). The use of field life-tables have been made recently for studying the natural population of insect pests. When the environmental parameters are related to several causes of mortality, the field life-tables form a budget of the successive process that operate in a given population. Field life-table studies indicate which age interval and independent variable should be studied in detail for the effective control of the pest. It is also important to grasp the real situation of seasonal prevalence of an insect pest for planning its successful control (Harcourt 1966, 1969; Morris and Millar 1954; Singh *et al* 1977).

2. Materials and methods

A non replicated trial was conducted in two trials comprising of 100 quadrates of $2{\cdot}4 \times 2{\cdot}1$ m size. The castor, VI-9 was raised during 'Kharif' season on main farm, College of Agriculture, Parbhani during the years 1985–86 and 1986–87. Frequent field visits were made in order to record the first incidence of (egg stage) *Achaea janata* on castor crop. The known number of eggs were collected and reared till pupation in laboratory and was continued till the cessation of pest in field. This culture was used as a check for further studies.

To decide the generations of pest regular sampling was taken based on the developmental stages of pest in check culture. Known number of sample quadrates

*Part of Ph.D thesis submitted by the first author.

were observed in early and late instar. The plants from sampled quadrates were observed for the presence of larvae and such 5 quadrates were observed per meteorological week. These collected larvae were reared in laboratory till pupation and observations were recorded on mortality of larvae and pupae due to different parasites and unknown reasons. An interval of 6 days was provided before next sampling for next generation after the emergence of previous adults. This period was considered as pre-oviposition act by moths. Known number of eggs were collected per generation and mortality owing to sterility and parasite was worked out.

The column headings used in preparation of field life-tables study were those proposed by Morris and Millar (1954) and Harcourt (1963, 1969).

x = The age interval, egg, larva, pupa and adult.
l_x = The number surviving at the beginning of stage noted in 'x'.
d_x = The number dying within the age interval stated in 'x'.
d_xF = The mortality factor responsible for 'd_x'.
100_{qx} = Percentage mortality.
S_x = Survival rate within the age mentioned in 'x'.

Separate budget for each generation was prepared to find out the key factor that influenced the population trend in different generation. The method for key factor analysis developed by Varley and Gradwell (1965), was used. By this method, the killing power (K) was estimated. As a series of mortality factor operated in succession during a generation of population, the total killing power of K was equal to the sum of the killing power of K's.

3. Results and discussion

The results obtained on the key mortality factors during the years 1985–86 and 1986–87 are presented in tables 1–8 and figure 1. It is evident from table 1 and

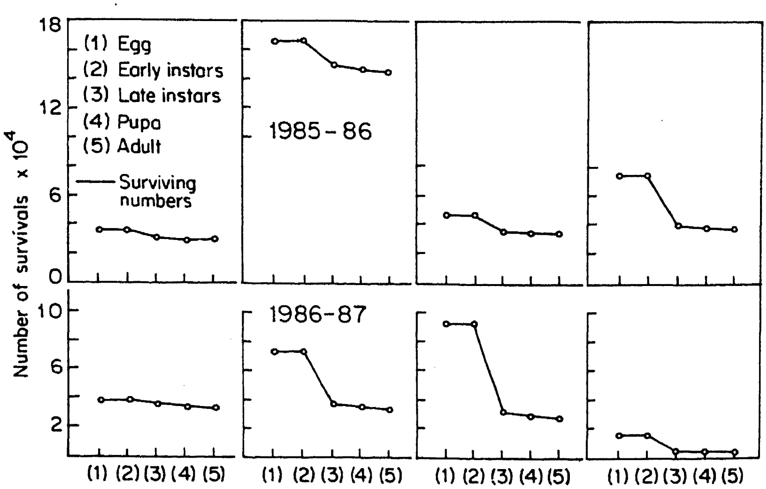

Figure 1. Survivorship curve of *A. janata* during 4 generations of castor (1985–86 and 1986–87).

Table 1. Key mortality factors for first generation of *A. janata* on castor during 1985–86 and 1986–87 population/ha.

Age interval (x)	No. alive at the beginning of x (l_x)	Factors responsible for d_x (d_xF)	No. dying during x (d_x)	d_x as a per cent of x (100_{qx})	Survival rate (S_x)
Expected eggs					
1985–86	43,823	Sterility/dead	6,573	14·99	0·85
1986–87	46,176		6,926	11·99	0·85
Viable eggs					
1985–86	37,250	—	—	—	—
1986–87	39,250	—	—	—	—
Larval instars					
Early instars					
1985–86 (I–III)N_1	37,250	*M. maculipennis*	500	1·34	0·87
1986–87	·39,250	–"–	—	—	—
1985–86	—	Unknown causes	4,125	11·07	—
1986–87	—	–"–	2,250	5·73	0·94
Late instars (IV–V)					
1985–86	32,625	–"–	1,250	3·83	0·96
1986–87	37,000	–"–	1,750	4·73	0·95
Pupae					
1985–86	31,375	–"–	625	2·00	0·98
1986–87	35,250	–"–	750	2·13	0·98
Moths					
1985–86	30,750	50% females	—	—	1·00
1986–87	34,500	–"–	—	—	1·00
Females × 2 (N_3)					
1985–86	30,750	Reproducing females	15,375		
1986–87	34,500	–"–	17,250		
Actual No. of younger larvae in next generation (N_2)					
1985–86	1,67,875				
1986–87	17,375				
Trend index (I)					
1985–86	4·506				
1986–87	1·869				
Generation survival (SG) N_3/N_1					
1985–86	0·82				
1986–87	0·87				

figure 1 that there was 14·38 and 11·99% mortality among the eggs owing sterility. The larval mortality because of parasite *Microplitis maculipennis* (szep) (Braconidae: Hymenoptera) was 1·34 and 0%, similarly larval mortality due to unknown causes was 11·07, 3·83 and 5·73, 4·73% in the first generation. The generation survival value (0·82 and 0·87) and the positive value of trend index (4·506 and 1·869) indicated that the mortality factors operated during first generation were not effective in causing a decline in population in the next generation. Table 2 indicates

Table 2. Budget of *A. janata* during first generation, 1985–86 and 1986–87.

Age interval	Number/ha 1985–86	Number/ha 1986–87	Log number/ha 1985–86	Log number/ha 1986–87	K's 1985–86	K's 1986–87
Expected eggs	43,823	46,176	4·6417	4·6644	—	—
Viable eggs	37,250	39,250	4·5711	4·5938	0·0706	0·0706
Actual early instar larvae after mortality due to parasite and unknown causes	37,250	39,250	4·5711	4·5938	0·0000	0·0000
Late instar after mortality due to unknown causes	32,650	37,000	4·5138	4·5682	0·0573	0·0256
Pupae after mortality due to unknown causes	31,375	35,250	4·4965	4·5471	0·0173	0·0211
Moths	30,750	34,500	4·4878	4·5378	0·0087	0·0093
Reproducing females	15,375	17,250	4·1868	4·2367	0·3010	0·3011
				Total K =	0·4549	0·4277

that the maximum mortality occurred in the early instar larvae of first generation with the highest K value (0·0573) and (0·0256). The number of reproducing females/hectare in first generation were 15375 and 17250 which actually contributed for population build up in the second generation.

It is evident from table 3 and figure 1 that there was 11·39 and 11·39% egg mortality due to sterility. The parasitization of larvae because of *M. maculipennis* was 4·91 and 44·12%. The generation survival values were 0·88 and 0·47. The negative value of trend index (0·301) indicated that the mortality factors operating during this period were effective in checking the population in the next generation for the year 1985–86 and it was vice versa for the year 1986–87. The early instar larvae had highest mortality ($K = 0{\cdot}0435$) and ($K = 0{\cdot}2988$) in the second generation (table 4, figure 1). The next important mortality factor was in pupal stage. At the end of second generation, 73875 and 17312 females were expected to contribute for population build up in the third generation.

It is observed from table 5 and figure 1 that the egg mortality due to sterility was 10·49 and 10·49 in the third generation. The mortality of early instar larvae by parasite was 22·02 and 61·46%. Total mortality in larval and pupal stages due to unknown causes was 4·05 and 16·14%. The generation survival value were 0·74 and 0·30. The trend index values (1·583 and 1·159) indicated that the mortality factors (major parasite) operating during this period were not effective in suppressing the pest population on castor. The age specific key mortality factors indicates that maximum contribution towards generation mortality came from the early instar larvae ($K = 0{\cdot}1179$ and $K = 0{\cdot}4753$) (table 6, figure 1). At the end of the third generation 17750 and 14250 females were expected to contribute for population build up in the fourth generation. The total K's for all the life stages was 0·4772 and 0·8793.

It is evident from table 7 and figure 1 that the mortality of eggs due to sterility was 13·99%. The parasite *M. maculipennis* parasitized the early instar larvae to the extent of 43·70 and 65·55% in fourth and last generation of the 1985–86 and 1986–87. The generation survival value was 0·52 and 0·26 and the trend index value remained zero in the last generation, hence the pest population was totally ceased. The

Table 3. Key mortality factors for second generation of *A. janata* on castor during 1985–86 and 1986–87 population/ha.

Age interval (x)	No. alive at the beginning of x (l_x)	Factors responsible for d_x (d_xF)	No. dying during x (d_x)	d_x as a per cent of x (100_{qx})	Survival rate (S_x)
Expected eggs					
1985–86	1,89,475	Sterility/dead	21,600	11·39	0·88
1986–87	82,816	–"–	9,441	11·39	0·36
Viable eggs					
1985–86	1,67,875	—	—	—	—
1986–87	73,375	—	—	—	—
Larval instar Early instars (I–III) N_1					
1985–86	1,67,875	*M. maculipennis*	8,250	4·91	0·90
		Unknown causes	7,750	4·61	—
1986–87	73,375	–"–	32,375	44·12	0·50
Late instar (IV–V)					
1985–86	1,51,875	–"–	3,125	2·05	0·98
1986–87	36,875	–"–	2,125	5·76	0·94
Pupae					
1985–86	1,48,750	–"–	1,000	0·67	0·99
1986–87	34,750	–"–	125	0·36	0·99
Moths					
1985–86	1,47,750	50% females	—	—	1·00
1986–87	34,625	–"–	—	—	1·00
Female × 2(N_3)					
1985–86	1,47,750	Reproducing females	73,875		
1986–87	34,625	–"–	17,312		
Actual No. of younger larvae in next generation (N_2)					
1985–86	4,766				
1986–87	3,833				
Trend index (I) = N_2/N_1					
1985–86	0·301				
1986–87	1·278				
Generation survival (SG) = N_3/N_1					
1985–86	0·88				
1986–87	0·47				

Table 4. Budget of *A. janata* during second generation, 1985–86 and 1986–87.

	Number/ha		Log number/ha		K's	
Age interval	1985–86	1986–87	1985–86	1986–87	1985–86	1986–87
Expected eggs	1,89,475	82,816	5·2775	4·9191	—	—
Viable eggs	1,67,875	73,375	5·2249	4·8655	0·0526	0·0526
Actual early instar larvae after mortality due to parasite and unknown causes	1,67,875	73,375	5·2249	4·8655	0·0000	0·0000
Late instars after mortality due to unknown causes	1,51,875	36,875	5·1814	4·5667	0·0435	0·2988
Pupae after mortality due to unknown causes	1,48,750	34,750	5·1724	4·5409	0·0090	0·0258
Moths	1,47,750	34,625	5·1695	4·5393	0·0029	0·0016
Reproducing females	73,875	17,312	4·8684	4·2383	0·3011	0·3010
				Total K=	0·4091	0·6798

Table 5. Key mortality factors for third generation of *A. janata* on castor during 1985–86 and 1986–87 population/h.

Age interval (x)	No. alive at the beginning of x (l_x)	Factors responsible for d_x (d_xF)	No. dying during x (d_x)	d_x as a per cent of x (100_{qx})	Survival rate (S_x)
Expected eggs					
1985–86	53,258	Sterility/dead	5,592	10·49	0·89
1986–87	1,04,841		11,008	10·49	0·89
Viable eggs					
1985–86	47,666	—	—	—	—
1986–87	93,833	—	—	—	—
Larval instars					
Early instars (I–III) (N_1)		*M. maculipennis*			
1985–86	47,666	Unknown	10,500	22·02	0·76
1986–87	93,833	causes	57,666	61·46	0·34
Late instars (IV–V)					
1985–86	36,333	–"–	333	0·92	0·99
1986–87	32,334	–"–	3,167	9·79	0·90
Pupae					
1985–86	36,000	–"–	500	1·39	0·99
1986–87	29,167	–"–	667	2·29	0·99
Moths					
1985–86	35,500	50% females	—	—	1·00
1986–87	28,500	–"–	—	—	1·00
Female × 2(N_3)					
1985–86	35,500	Reproducing	17,750		
1986–87	28,500	females	14,250		
Actual number of younger larvae in next generation (N_2)					
1985–86	75,500				
1986–87	15,000				

(*Contd.*)

Table 5. (*Contd.*)

Age interval (x)	No. alive at the begining of x (l_x)	Factors responsible for d_x (d_xF)	No. dying during x (d_x)	d_x as a per cent of x (100_{qx})	Survival rate (S_x)
Trend index (I) = N_2/N_1					
1985–86	1,583				
1986–87	1,159				
Generation survival (SG) = N_3/N_1					
1985–86	0·74				
1986–87	0·30				

Table 6. Budget of *A. janata* during third generation, 1985–86 and 1986–87.

	Number/ha		Log number/ha		K's	
Age interval	1985–86	1986–87	1985–86	1986–87	1985–86	1986–87
Expected eggs	53,258	1,04,841	4·7263	5·0205	—	—
Viable eggs	47,666	93,833	4·6782	4·9723	0·0481	0·0482
Actual egg early instar larvae after mortality due to parasite and unknown causes	47,666	93,833	4·6782	4·9723	0·0000	0·0000
Late instar after mortality due to unknown causes	36,333	32,334	4·5603	4·5096	0·1179	0·4753
Pupae after mortality due to unknown causes	36,000	29,167	4·5563	4·4648	0·0040	0·0448
Moths	35,500	28,500	4·5502	4·4548	0·0061	0·0100
Reproducing females	17,750	14,250	4·2491	4·1538	0·3011	0·3010
				Total K =	0·4772	0·8793

budget for fourth generation in which the mortality factor in larval stage was $K=0{\cdot}2705$ and $K=0{\cdot}5228$. The value of total K remained 0·6522 and 0·9405 for all the life stages (table 8, figure 1).

Khan (1946) reported 5 to 6 generations of *A. janata* on castor, the second instar larvae were attacked by *M. maculipennis* parasite in Andhra Pradesh. Five overlapping generations have been reported by Pandey *et al* (1967) on castor. According to Srivastava and Pande (1966), *Achaea* completed 3 generations in Bikaner–Barmer tract and 5 generations in Ajmer–Bharatpur tract of Rajasthan. Cherian and Basheer (1946) observed 56·2% parasitization of *A. janata* larvae by *M. maculipennis*. The larval parasitization was to the extent of 70–75% on castor in Karnataka (Rai and Jayaramaiah 1978). The results obtained in the present investigation in relation to generation and per cent parasitization are in agreement with the earlier workers with little variation (Khan 1946; Srivastava and Pande 1966; Pandey *et al* 1967; Rai and Jayaramaiah 1978).

From these studies it has been observed that the per cent parasitization of *M. maculipennis* exhibited an increasing trend in both the years and reached at its peak in the last generation. The high per cent parasitization resulted suppressing

Table 7. Key mortality factors for fourth generation of *A. janata* on castor during 1985–86 and 1986–87 population/ha.

Age interval (x)	No. alive at the beginning of x (l_x)	Factors responsible for $d_x(d_xF)$	No. dying during x (d_x)	d_x as a per cent of x (100_{qx})	Survival rate (S_x)
Expected eggs					
1985–86	87,790	Sterility/dead	12,290	13·99	0·86
1986–87	17,441		2,441	13·99	0·86
Viable eggs					
1985–86	75,500	—	—	—	—
1986–87	15,000	—	—	—	—
Larval instars					
Early instars (I–III) N_1		*M. maculipennis*	33,000	43·70	0·54
1985–86	75,500	Unknown causes	2,000	2·65	—
1986–87	15,000	–"–	9,833	65·55	0·30
Late instars (IV–V)					
1985–86	40,500	–"–	875	2·16	0·98
1986–87	4,500	–"–	167	3·71	0·98
Pupae					
1985–86	39,625	–"–	250	0·63	0·99
1986–87	4,333	–"–	333	7·76	0·92
Moths					
1985–86	39,375	50% females	—	—	1·00
1986–87	4,000	–"–	—	—	1·00
Females × 2(N_3)					
1985–86	39,375	Reproducing females	19,687·5		
1986–87	4,000	–"–	2,000		
Actual No. of younger larvae in next generation (N_2)					
1985–86	Nil				
1986–87	Nil				
Trend index (I) $= N_2/N_1$					
1985–86	Nil				
1986–87	Nil				
Generation survival (SG) $= N_3/N_1$					
1985–86	0·52				
1986–87	0·26				

Table 8. Budget of *A. janata* during fourth generation, 1985–86 and 1986–87.

	Number/ha		Log number/ha		*K's*	
Age interval	1985–86	1986–87	1985–86	1986–87	1985–86	1986–87
Expected eggs	87,750	17,441	4·9434	4·2415	—	—
Viable eggs	75,500	15,000	4·8750	4·1760	0·0684	0·0655
Actual early instar larvae after mortality due to parasite and unknown causes	75,500	15,000	4·8750	4·1760	0·0000	0·0000
Late instar after mortality due to unknown causes	40,500	4,500	4·6074	3·6532	0·2705	0·5228
Pupae after mortality due to unknown causes	39,625	4,333	4·5952	3·6367	0·0095	0·0165
Moths	39,375	4,000	4·5952	3·6020	0·0027	0·0347
Reproducing females	19,687	2,000	4·2941	3·3010	0·3011	0·3010
				Total K =	0·6522	0·9405

the pest population in the next generation, hence this parasite could be considered for the management programme of *A. janata* on castor.

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Maturity and breeding of the mud crab, *Scylla serrata* (Forskal) (Decapoda: Brachyura: Portunidae)

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Abstract. Females of *Scylla serrata* attained sexual maturity after reaching 80 mm carapace width and above. Fifty per cent of females at size range 91–100 mm carapace width were sexually mature. The growth rate of abdomen width or length with respect to either carapace width or length was generally at much higher side in mature females than in the immature specimens. A sharp transition at 80 mm carapace width indicated the morphological changes accompanied with the sexual maturity. The relationship between abdomen allometry and carapace measurements was highly significant ($P < 0{\cdot}001$). *Scylla serrata* although bred all through the year, exhibited two peaks of breeding, one in between December–March and another in September–November. The sex-ratio in two biotopes varied considerably with the breeding season and size of the female population. The females were found to be in berried state in inshore and off-shore waters of Karwar at 90 mm carapace width and above. Spawning of *Scylla serrata* was found to occur only in inshore and off-shore but not in backwaters.

Keywords. Maturity; breeding; mud crab; allometry; biotopes; berried.

1. Introduction

There are several studies on reproductive biology of portunids inhabiting Indian aquatic biotopes (Naidu 1955; Chhapgar 1956; Pillay and Nair 1968, 1971; Joel and Raj 1980) but the estuarine mud crab *Scylla serrata* remains as a topic of interest due to its unique spawnal migration. Throughout its occurrence in Indo-Pacific range, ovigerous females form a significant and regular components of the fishing catch (Arriola 1940). Incidental catches of ovigerous *S. serrata* are also reported by trawlers operating in deep waters of Malaysia (Ong 1966). Although the study of occurrence of ovigerous females in commercial fish catches forms a direct method to indicate the spawning seasons, detailed gonadal examination is necessary to depict various reproductive aspects.

The present work therefore is an attempt to obtain the knowledge of the breeding seasons and breeding grounds of *S. serrata* off Karwar. Stages of maturity, size at maturity and sex-ratio of *S. serrata* population are also dealt with.

2. Materials and methods

Crabs were collected from backwaters (from long lines) and inshore waters (gill-nets, shore-seines and trawlers) of Karwar (14° 46′ 54″ N and 74° 03′ 00″ E to 14° 54′ 25″ N and 74° 19′ 30″ E) during December 1984 and November 1985. The crabs were sexed and the live weight, adjusted whenever necessary for missing periopods, was measured to the nearest gram.

2.1 *Ovary examination*

To facilitate the removal of ovary, a cut was made in the carapace along and just ventral to the antero-lateral spines allowing the carapace easily to be lifted off exposing the ovary and other internal organs. The gonado-somatic index (GSI) (drained ovary weight as a percentage of total body weight) was then calculated for each crab.

2.2 *Maturity stages*

Colour changes in the ovary and oocyte diameter were taken into consideration to classify the maturity stages (Wild 1983). The size of the ovary with respect to space occupied in haemocoel (Shanmugam and Bensam 1980) and GSI were also considered (Haesman 1980) in the classification. The following 4 stages of development in the ovary were observed:

Stage I: The ovary is white, thin, transparent and thread like occupying 1/10th of the body cavity. There is no prominent seminal receptacle. The ova diameter ranges from 0·01–0·06 mm with a modal value of 0·05 mm. The GSI is very low and is up to 0·5.

Stage II: It shows the peripheral undulations for the formation of ovarioles. It represents the partial development of ovaries and occupying about 1/5th of the haemocoel. Colouration of the ovary ranges between yellowish to pinkish. The ova diameter varies from 0·10–0·30 mm with a modal value of 0·20 mm. The GSI ranges from 0·5–1·5.

Stage III: The stage denotes the maturing condition of the ovaries extending into anterolateral region of the carapace thereby occupying about half of the body cavity. The ovaries are orange in colour and the ova diameter ranges between 0·40 and 0·90 mm with a modal value of 0·80 mm. The GSI ranges from 2·5–8·0.

Stage IV: This stage shows the intensification of the colour (bright red) depicting the rapid maturation of the oocyte. There is a well defined seminal receptacle. The ovaries occupy approximately more than 3/4th of the haemocoel. The ova diameter ranges from 0·70–1·30 mm with a modal value of 1·15 mm. The GSI is as high as 15·85.

Spent stage is indicated by a flacid, speckled appearance of ovaries resulting from aggregation of unspawned ova. Ova colour ranges from red to brown and distinguished with the fawn to grey shrunken ovarian lobules.

Among ovigerous females two stages in the development of eggs could be recognised, one in which the eggs attached to abdominal appendages are orange red in colour and the other are dark brown representing more advanced stage.

2.3 *Size at first maturity*

Size at first maturity was determined by tabulating the percentage of crabs in different stages against size (carapace width).

2.4 *Morphometric measurements*

Certain morphometric changes often accompany the pubertal moult in brachyuran crabs (Haley 1973; Hartnoll 1974). During the puberty the shape and dimensions of abdomen in female crabs are greatly changed and are modified. The morphometric characters were made using dial calipers to determine whether any relation existed between the morphology and sexual maturity.

2.5 *Breeding*

The gonadal observations were initially carried out on large number of *S. serrata* including all the moulting stages. Later, however, only 1519 intermoult (hard shelled) adult crabs (> 80 mm carapace width) were sacrificed to determine the breeding periodicity, as soft shelled and small sized crabs yielded very eratic information regarding the breeding state of the animal. Besides tabulating mean GSI every month, a periodic incidence of ovigerous females were recorded to know the status of breeding population. The breeding grounds were inferred from the place of incidence of sponge bearing crabs with dark brown eggs—a stage which is indicative of actual hatching time (Joel and Raj 1980).

2.6 *Sex-ratio*

Looking at the shape of the abdomen a total of 7953 crabs were sexed in the commercial catches, without sacrificing them, to throw some light on population structure.

2.7 *Statistical analysis*

Morphometric data were plotted and the regression equations were calculated by assuming an allometric growth equation $Y = a + bX$, where X = carapace width or carapace length as the case may be and Y the variable being measured (Snedecor and Cochran 1967).

3. Results

3.1 *Size at maturity*

In Karwar waters, females of *S. serrata* attained sexual maturity only after reaching 80 mm carapace width. The proportion of immature (stage I), maturing (stages II and III) and mature (stage IV) stages of ovary within each size range varied between backwaters and inshore waters. The percentage of maturity increased with the size till 141 mm but thereafter it was irregular and the crabs belonging to 181–190 mm size range were found to be sexually active indicating high percentage of maturity. In both backwaters and inshore waters 50% of female crabs at size range 91–100 mm were mature (figure 1).

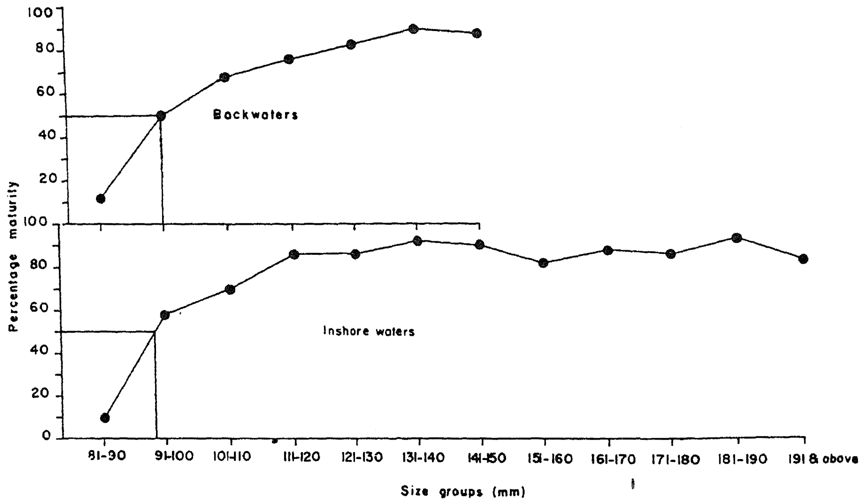

Figure 1. Percentage of maturity of females indicating 50% size at maturity.

3.2 *Morphometric characters*

The allometric growth equations of morphometric measurements along with correlation coefficient (r) values are indicated in figure 2.

The growth rate of abdomen width with respect to carapace width was lesser ($b = 0{\cdot}6870$) in mature specimens than that of immature ones ($b = 0{\cdot}7350$). The growth rate of abdomen width or length was generally at higher side in mature crabs than in the immature specimens. The sharp break or transition at about 80 mm size indicated the occurrence of morphological changes associated with maturity (figure 2). The morphological relationships in both immature and mature crabs were highly significant at $P < 0{\cdot}001$.

3.3 *Breeding*

Monthly observations of gonadal development depicted the prolific breeding nature of *S. serrata* possessing all maturity stages throughout the year but with a considerable seasonal variation. Mean GSI values exhibited two peaks—one between December–March and another between September–November (figure 3). Correspondingly the observations confirmed the maximum incidence of ovigerous females during the same period (figure 4). Hence these seasons were considered as the actual breeding seasons. Environmental parameters such as salinity and temperature during the period of study indicated that high GSI and incidence of mature stages (IV) in female crab population coincided with fairly moderate salinity and temperature (figure 5).

The analysis of sex-ratio of crabs from backwaters and inshore waters showed that although there was near equal proportion of males and females in both the biotopes, the sex-ratio varied considerably with the season and size of female population (Prasad 1987). The proportion of females particularly adults dropped considerably in the backwaters during peak breeding seasons (October–December) and the same had increased in the inshore water population. This drop was

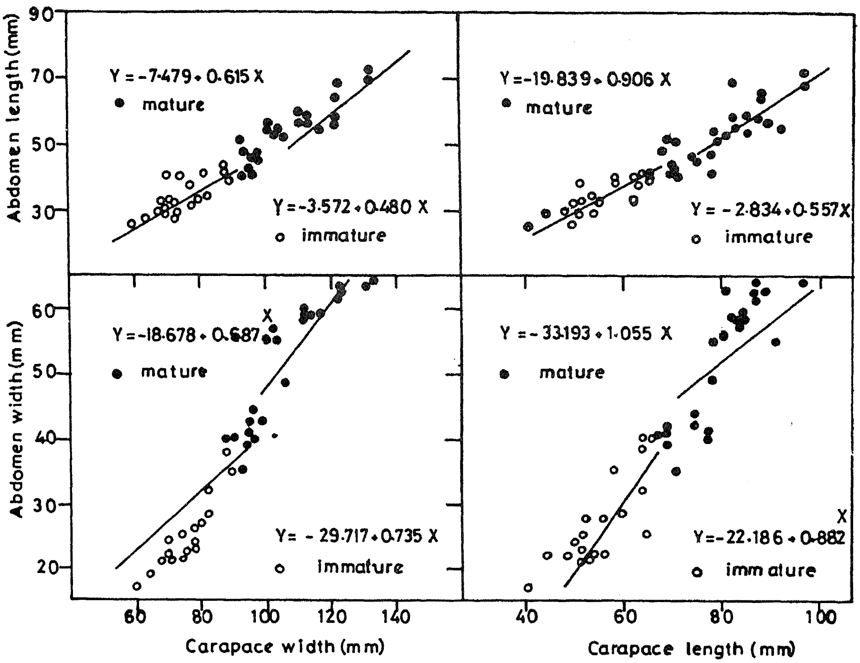

Figure 2. Relationship between abdomen allometry and carapace measurements.

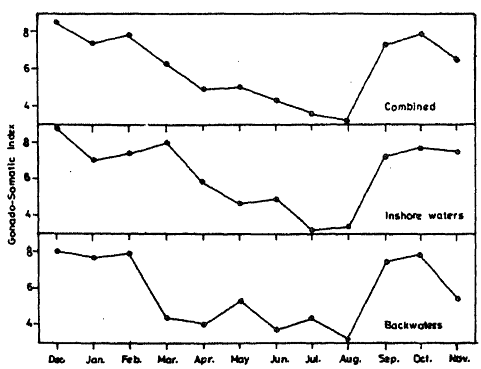

Figure 3. Monthly variation in GSI values in female crabs during Dec. 1984 and Nov. 1985.

probably be due to migration of female crabs out of backwaters of Kali estuary into the sea. The mating processes however appeared to take place both in backwaters and inshore waters as the mating pairs (soft shelled female and hard shelled male) were caught in both the biotopes. Nevertheless the females were found to get into berried state only in the sea. The berried females were never caught by long lines in backwaters during the entire duration of the study. Further majority (52·05%) of the berried crabs caught belonged to trawl catch which came from nearshore and offshore waters of Karwar. Although female crabs tended to get into berried form at 90–100 mm size itself, it was actually 120–130 mm group found to be active contributing a larger share (31·58%) to the berried females caught in Karwar

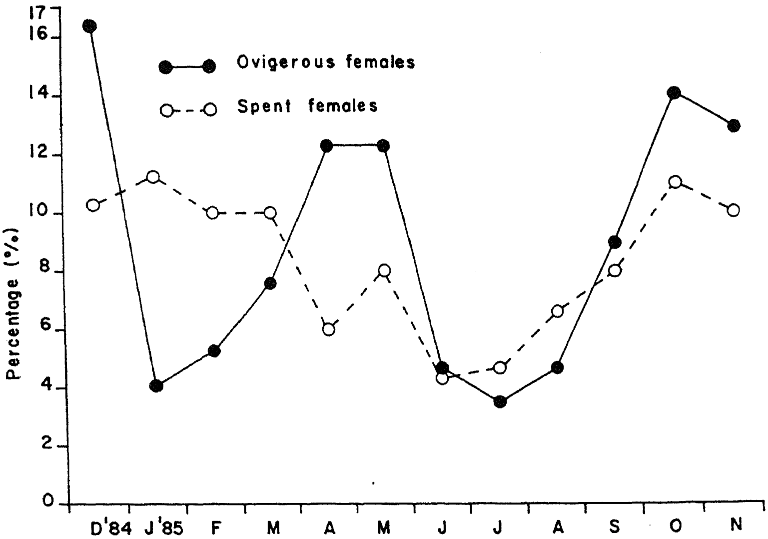

Figure 4. Incidence of ovigerous and spent females of *S. serrata* in Karwar waters.

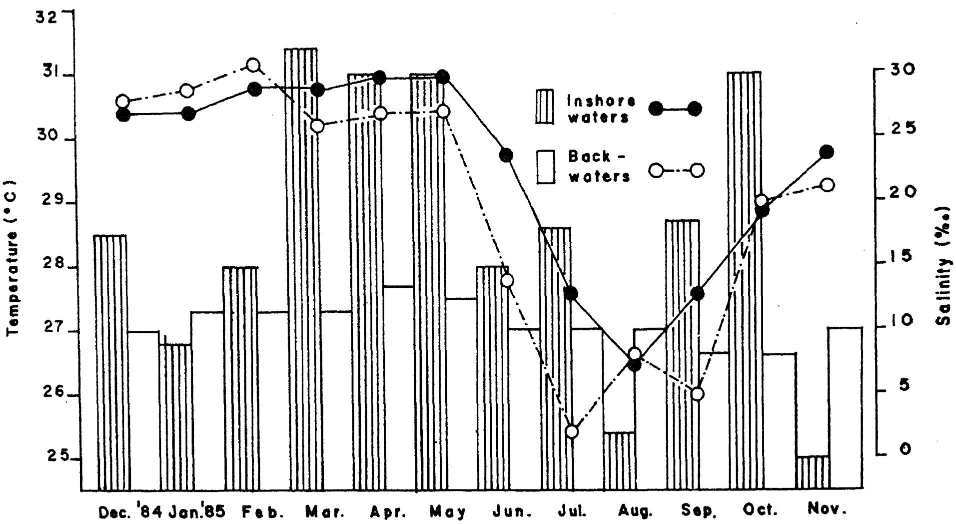

Figure 5. Seasonal variation of salinity and temperature in Karwar waters.

waters. Out of 1519 adult females examined, 19·81% (N=301) of crabs were identified as spent females but majority (N=219) of them were caught by gill-nets and shore-seines in inshore waters.

4. Discussion

4.1 *Size at maturity*

It is reported that the fresh water female crab *Barytelphusa cunicularis* becomes

sexually mature on attaining a carapace width of about 44 mm (Diwan 1973) while in the case of *B. guarini* it was about 40 mm carapace width (Gangotri *et al* 1971). From the gonadal study it is evident that although *S. serrata* attains sexual maturity (with stage IV ovary) in 81–90 mm size range itself, the animals were found to be sexually active only with size range of 120–180 mm. Moreover, unlike that of males (Prasad 1987) the female crabs never attained absolute (100%) maturity at any given size range. The reproductive activity seemed to decline in older animals with a carapace width of 190 mm and above.

A small percentage of immature ovaries even in the crabs belonging to 120 mm and above further indicate that even larger crabs also had to be impregnated more than once for the development of ovary. The moulting stage, presence of seminal products in spermatheca (Ezhirarasi and Subramoniam 1980) and certain neurosecretory factors (John and Sivadas 1979) are of prime importance to initiate the development of ovaries. The photoperiod and nutrient reserves have also been attributed to the rate of development of ovaries in *S. serrata* (Nagabhushanam and Farooqui 1980).

4.2 *Morphometric characters*

It can be seen from figure 2 that morphometric measurements indicate differential post maturity growth pattern in females. However these growth patterns were of negative allometry showing the exponential values (b) less than the unity. It is generally agreed that although a sudden increment of these secondary sexual characters takes place at pubertal moult, majority of the female portunids showed negative allometry as the frequency of moulting decreases after puberty (Watson 1970; Hartnoll 1974). What is finally evident from the results is that the pubertal moult in female *S. serrata* occurs at 80 mm carapace width and above thereby bringing an abnormal increase in certain morphometric characters such as abdomen dimensions. The same is illustrated in figure 2 with a clear transition at puberty and gonadal observations corresponding with the fact that the females attain puberty only after reaching 80 mm carapace width. These morphometric characteristics may have some adoptive significance while providing sufficient space for fertilized eggs thereby creating a congenial incubation chamber.

4.3 *Breeding*

Chandran (1968) while working on breeding of marine crabs of Indian coast hypothesised that the salinity, temperature and ample supply of food material are some of the important factors which trigger the breeding mechanisms. Although the mud crab breeds continuously throughout the year in Karwar waters, it had two distinct peaks one in December–March and another in September–November when the salinity and temperature were neither high nor low (figure 5). These conditions seemed to be ideal for successful incubation and larval development.

Haesman *et al* (1985) while presenting a review of general trends of spawning activity of *S. serrata* has concluded that the length of the spawning period increases with the decreasing latitude. One of the reasons accounted was the availability of food in differential amounts in different seasons. For Indian waters Pillay and Nair

(1968) drew attention to the possible influence of post monsoonal upwelling on t peaks of spawning during November–January in southwest coastal waters.

The available information on spawning migrations of *S. serrata* has be reviewed by Hill (1975). It was noticed that spawning migrations usually follow lunar cycle and also salinity changes. Several reports reveal that *S. serrata* spaw in the sea and the young ones migrate either to inshore waters or backwate Distance travelled in the spawning migrations however greatly vary in accordan with topographical features and prevailing hydrological conditions. Ovigero females in shallow (1–15 m) lagoons, bays, inlets and coastal waters have be generally captured in Philippines (Arriola 1940), Hawaii (Brick 1974) and Inc (Naidu 1955). Joel and Raj (1980) have noticed the berried females of *S. serrata* brackish water zones of Pulicat lake mouth adjacent to sea. The incidence sponge bearing females in Karwar waters indicated that spawning grounds of *serrata* are located in both inshore and off-shore up to 20–25 km. Majority of t ovigerous females (52·05%) were caught in trawlers which were operated off t shore and as near as 8–25 km from the backwaters of Kali estuary. Interestingly c of 1915 female crabs examined in backwaters none of them was with a 'berry'. Su absence of ovigerous females in the backwaters could be primarily due to seawa spawnal migration of mature females. This view has been strengthened with hi sex-ratio (males/females) during the peak breeding season. Secondly ovigerc females, if any, may not have been accessible for 'long lines' a major gear employ in backwaters, for catching *S. serrata*, by the simple fact that the intensity of feedi in crabs decline during berried state (Edwards 1979; Haesman *et al* 1985). Thirdly could also be due to timidity or protective behaviour towards the eggs. conclusion therefore it may be said that the recruitment of *S. serrata* population Karwar waters takes place from the spawning grounds located in the inshore a off-shore waters of Karwar.

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Induction of allotriploids in the hybrids of *Oreochromis mossambicus* female × red* tilapia male

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Abstract. Cent percent allotriploids were induced by heat shocking (42°C for 3 min) of 2·5 min old (post-fertilization) *Oreochromis mossambicus* eggs fertilized with (homozygous) red tilapia milt. Control diploid hybrids were red (orange) in colour. A few triploid hybrids displayed more patches of black pigmentation. Hence red and black colours are regarded as co-dominants. Survival of the allotriploids was higher than that of *Oreochromis mossambicus* autotriploids.

Keywords. Allotriploidy; heat shock; codominant.

1. Introduction

In recent years induction of triploidy has been extensively attempted in fish (Donaldson and Benfey 1987). The results of most of these studies recall earlier ones on amphibians (e.g. Fankhauser 1945). Triploids are generally sterile owing to the probable failure of homologous chromosomes to pair precisely during meiosis (Thorgaard 1983). In tilapias, the major interest in triploidization is the production of sterile fish, in which the undesirable traits associated with gonadal maturation on growth of fertile diploid are avoided (Pandian and Varadaraj 1987).

Allotriploidy has already been reported for salmon (Chevassus *et al* 1983; Scheerer and Thorgaard 1983). Thorgaard (1983) suggested that hybrid triploids may have better survival than diploid hybrids, and that triploidization lead to increased viability in interspecific hybrids. As the major problem in the worldwide culture of tilapias is their uncontrolled reproduction (Hickling 1963; Lovshin 1982; Wohlfarth and Hulata 1983; Hulata *et al* 1981, 1983; Das *et al* 1987; Pandian and Varadaraj 1987), the need for the production of allotriploidy has become a necessity. Enhanced survival and growth of the allotriploids may boost tilapia aquaculture. This paper describes a technique for the induction of allotriploidy in tilapias.

2. Materials and methods

Oreochromis mossambicus (brownish black colour) (♀) and red colour tilapia (♂) stocked in indoor tanks served as the source of eggs and milt. The eggs were stripped from 3 fully mature females. They were immediately wet fertilized with milt obtained from *O. mossambicus* or red tilapia. One batch of eggs fertilized with *O. mossambicus* milt served as control 1 and the second fertilized with red tilapia milt as control 2. To ensure induction of 100% triploidy, one has to determine precisely

*The fish is orange in colour, however it is cited as red tilapia as is commonly known.

the time of second meiotic metaphase, and the effective level and duration of thermal shock for the successful retention of second polar body; these characteristics are species specific (Streisinger *et al* 1981) and in some cases even race specific (Thorgaard 1983). In general, cold shock is known to induce ploidy in tropical fishes (Thorgaard and Allen 1987; John *et al* 1984); however, Pandian and Varadaraj (1987) and Varadaraj and Pandian (1988) have shown that *O. mossambicus* is more amenable to heat rather than cold shock; they reported that autotriploidy can be induced in *O. mossambicus* by exposing 2·5–min old eggs to 42°C for 3 min. Therefore, in the present study, the ranges of temperature and duration of heat shock were restricted to 39–42°C and 1–5 min respectively. For the heat shock treatment, the eggs fertilized by red tilapia sperm at 29 ± 1°C were divided into 4 groups (100 each) based on post-fertilization age, viz. 1·0, 2·5, 3·5 or 4·5 min after insemination (see table 1). Eggs retained in plastic containers (7 cm dia and 3 cm depth with a screen of 1000 μm mesh at the bottom) were exposed to hot water at 39, 40 or 42°C for the duration of 2, 3 or 5 min at each of the tested temperatures.

After the treatment, the eggs were transferred to a plastic tray filled with sterilized tap water to regain their pre-shock temperature (29 ± 1°C) and incubated over a shaker until they hatched. Survival of the embryo/fry was monitored. Chromosome preparations were made from the gill tissue of control (50 numbers) and all the experimental groups using the air-drying method (Klingerman and Bloom 1977). The sex of the fish was determined by gonadal examination. Body pigmentation of fry and fingerling was examined with a light microscope.

3. Results and discussion

Cent percent allotriploidy could successfully be induced by heat shocking 2·5-min old fertilized eggs at 42°C for a duration of 3 min and the survival of embryos was 85%. About 6% of the induced triploids were deformed (table 1).

The metaphase spreads of diploid control 1 ($2n = 44$), diploid hybrid control 2 ($2n = 44$) and triploid hybrid ($3n = 66$) are shown in figure 1. They confirm the successful induction of allotriploidy. Chromosome preparations indicated the presence of a pair of large subtelocentric chromosomes both in the diploid control and hybrid diploid control. However, 3 subtelocentric chromosomes were observed of the hybrid triploid and were similar to that of the autotriploid. The large subtelocentric chromosome can serve as a marker for determination of ploidy. Neither haploid nor mosaic, as reported by Swarup (1959) for *Gasterosteus aculeatus*, was observed in the present study. It is worth noting that hybridization of *O. mossambicus* with red tilapia did not result in spontaneous triploidization, as it occurs in the hybrids of ♀ coho salmon × ♂ brook trout (Uyeno 1972) and ♀ rainbow trout × ♂ brook trout (Capanna *et al* 1974).

Survival of the allotriploid *O. mossambicus* was significantly ($P < 0.001$) higher (85%) than that of the autotriploid (68%) (Pandian and Varadaraj 1988). Our results confirm the increased viability of allotriploids observed by Thorgaard (1983), Chevassus *et al* (1983) and Arai (1986). Since allotriploid tilapia has two sets of maternal chromosomes and one set of paternal chromosomes in the ooplasm (maternally transmitted) of the hybrid, a decrease of the ratio of paternally derived genes may contribute to the restoration of normal development, as described by Arai (1984, 1986).

Table 1. Ploidy induction by heat shock in hybrid between *O. mossambicus* (♀) and red tilapia (♂).

Thermal shock Temperature (°C)	Thermal shock Duration (min)	Time after insemination (min)	Survival (%)	Ploidy 2n (%)	Ploidy 3n (%)	Deformed fry (%)
Control 1	—	—	93	100	0	0
Control 2	—	—	91	100	0	0
39	2	1·0	89	100	0	0
	2	2·5	86	100	0	2
	2	3·5	82	100	0	0
	2	4·5	76	100	0	0
	3	1·0	85	100	0	3
	3	2·5	81	100	0	2
	3	3·5	78	100	0	0
	3	4·5	79	100	0	5
	5	1·0	80	100	0	0
	5	2·5	78	93	7	6
	5	3·5	74	100	0	2
	5	4·5	77	100	0	0
40	2	1·0	78	100	0	4
	2	2·5	82	100	0	3
	2	3·5	76	100	0	9
	2	4·5	79	100	0	3
	3	1·0	75	100	0	0
	3	2·5	79	95	5	6
	3	3·5	74	100	0	9
	3	4·5	78	100	0	2
	5	1·0	73	100	0	7
	5	2·5	70	86	14	11
	5	3·5	72	100	0	8
	5	4·5	69	100	0	2
42	2	1·0	76	97	23	7
	2	2·5	72	72	28	5
	2	3·5	78	95	5	9
	2	4·5	81	100	0	2
	3	1·0	83	94	6	0
	3	2·5	85	0	100	6
	3	3·5	78	79	21	2
	3	4·5	71	100	0	3
	5	1·0	0	0	0	0
	5	2·5	0	0	0	0
	5	3·5	0	0	0	0
	5	4·5	0	0	0	0

For each experimental schedule as well as control 5 replicates of 100 eggs each were used.

During development, conspicuous difference in pigmentation became apparent among normal (wild type) and hybrid (red colour) diploids, when they attained the yolksac and fry stages (figure 2). The difference in melanin pigmentation was so conspicuous that pigmentation may be considered as a marker to distinguish diploid hybrid from the allotriploids (figure 3). Compared to diploid, triploids display more patches of melanin pigmentation. Irrespective of sex, all the diploid hybrids were more or less red in colour (figure 4). Obviously the genes responsible for body colour are not sex-linked. However, males had a brownish red belly

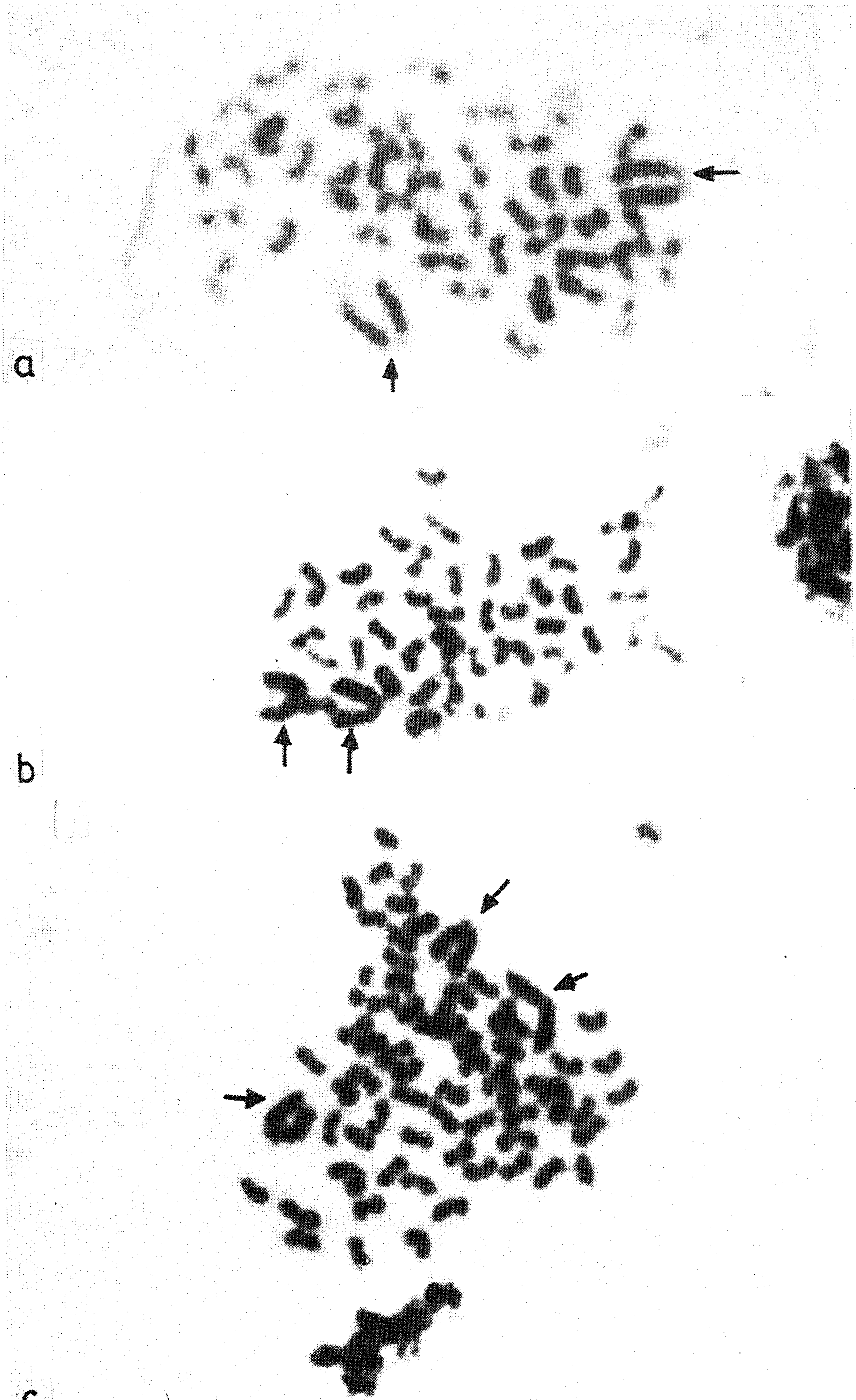

Figure 1. Metaphase spread of (**a**) diploid wild *O. mossambicus* fry (2n = 44), (**b**) diploid hybrid (2n = 44); (*O. mossambicus* ♀ × red tilapia ♂) and (**c**) triploid hybrid (3n = 66; *O. mossambicus* ♀ × red tilapia ♂). Arrows indicate marker chromosome.

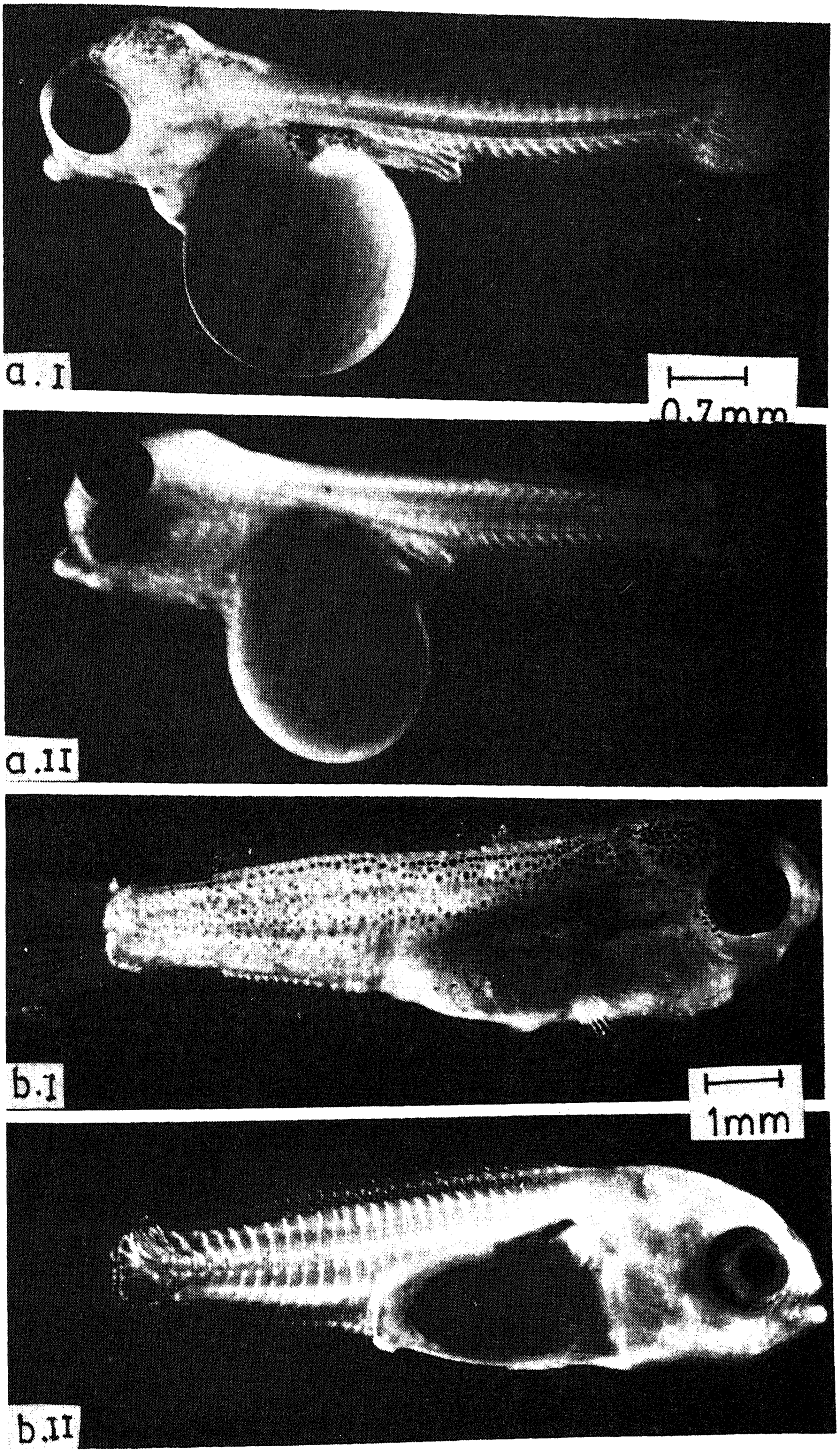

Figure 2. Melanin pigmentation. (**a**) Yolksac stage. (**b**) Fry stage. (I), Wild type; (II), hybrid.

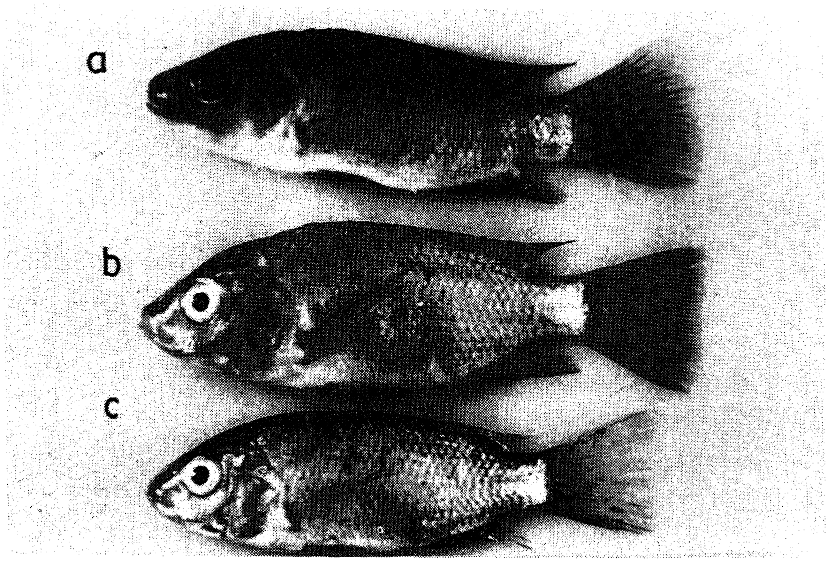

Figure 3. Morphological difference (body colour) in (**a**) wild type (*O. mossambicus*), (**b**) diploid hybrid (*O. mossambicus* ♀ × red tilapia ♂) and (**c**) triploid hybrid.

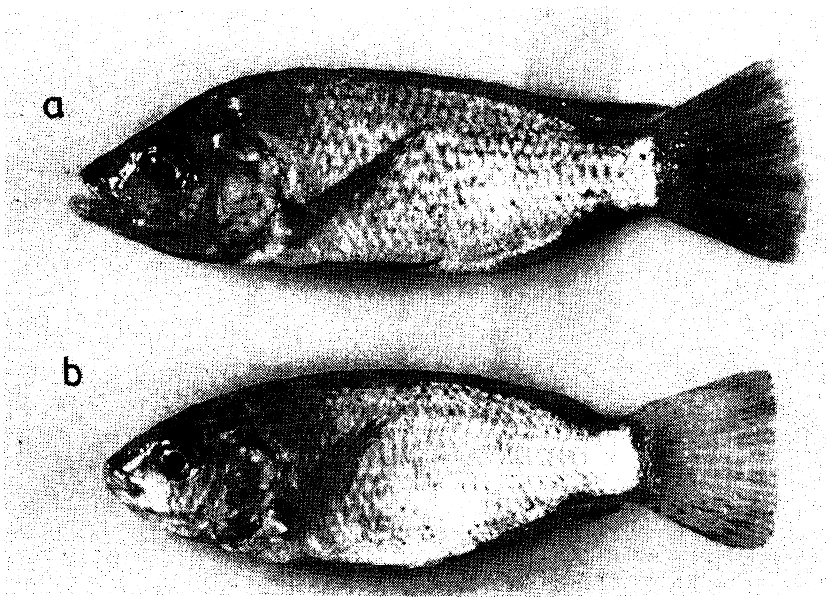

Figure 4. Morphological difference (colour) in diploid hybrid (**a**) male and (**b**) female offspring of *O. mossambicus* (♀) × red tilapia (♂).

whereas females had light yellow. Ventral region of the head in males was red, whereas that of females was dark yellow (figure 5). Available information on the inheritance of the red colour in red tilapia is confusing; for instance, Mires (1988) reported that the traits such as red and pink colour in *O. niloticus* are recessive to

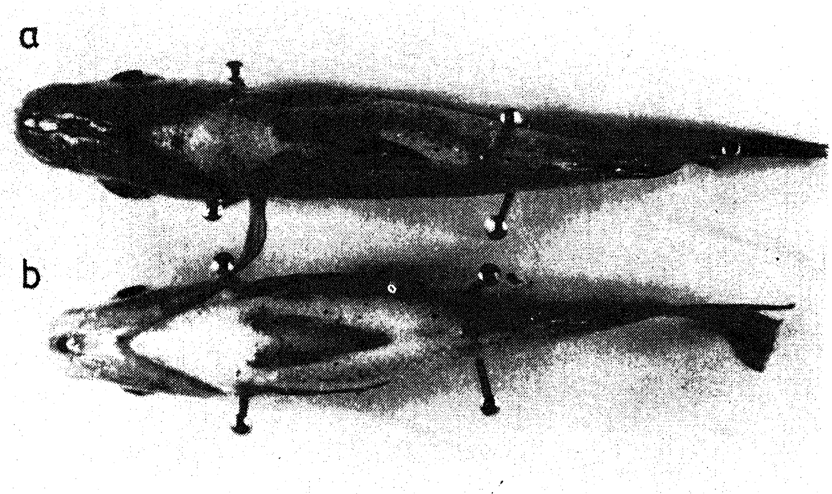

Figure 5. Colour difference in the ventral side of the (**a**) male (brownish red) and (**b**) female (dark yellow).

black. Conversely, Huang *et al* (1988) considered that the red trait is dominant over black in Taiwanese red tilapia. The occurrence of melanic patches on the hybrid diploid and triploid in the present study suggests that the traits responsible for red and black colours are rather codominant, as the gene for coat colour in cattle (Crockrum and McCauley 1965).

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Nitrogen budget of *Callosobruchus maculatus* larva developing in different host seed species

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Abstract. Studies on effect of seed species and their interacting influence on food utilization of *Callosobruchus maculatus* clearly pointed out that rates and efficiencies of nitrogen utilization are determined by the composition of the seed also. Of the 3 species of seeds tested, *Vigna unguiculata* has significantly more nitrogen and water per unit weight than the other two seeds, *Dolichos lab lab* and *Phaseolus radiatus*. With increasing nitrogen content of the seed, rates of feeding, assimilation and metabolism decreased. On the other hand efficiencies of assimilation and net production increased. Nitrogen assimilation efficiency of *Callosobruchus maculatus* ranged from 76·6% in *Dolichos lab lab* to 85·8% in *Vigna unguiculata*. The efficiency of accumulation of assimilated nitrogen was around 82%. The less nutritious *Dolichos lab lab* contributed more to the observed variance in the efficiencies of assimilation and production. The low efficiencies may be attributed to the presence of protease inhibitors.

Keywords. Nitrogen budget; *Callosobruchus maculatus*; *Dolichos lab lab*; *Phaseolus radiatus*; *Vigna unguiculata*.

1. Introduction

The pattern of food utilization and energy allocation to reproduction in insects is significantly influenced by humidity (Pingale 1976), food quality (Singh and Krishna 1980), temperature (White and Sinha 1981), life style (Cairns 1982) and interaction of one or more of these factors (Muthukrishnan and Pandian 1984). Scriber and Slansky (1981) showed that nitrogen and water content of food may significantly influence the assimilation efficiency of lepidopteran insects. The present observation pertains to the nitrogen budget of a granivorous insect *Callosobruchus maculatus* (F). feeding on different host seed species.

2. Materials and methods

2.1 *Estimation of nitrogen content*

Nitrogen budget of *C. maculatus* was studied as functions of host seed species, *Vigna unguiculata*, *Phaseolus radiatus* and *Dolichos lab lab*. Seeds were taken in 5 cm dia petridish. Newly emerged adults were allowed to oviposit on the seeds. Subsequent to oviposition, seeds with single egg were separated, weighed individually, numbered with marker and transferred to glass vials. Ten replicates of 200 seeds containing one egg each were maintained. Simultaneously, 100 seeds of more or less the same weight but without any egg were maintained in

petridish as control. To ascertain hatching and moulting to higher instar, a minimum of 5 infested seeds from each replicate were dissected everyday. The presence of exuvia indicated moulting to the next instar. On the day of moulting to the next instar, unfed seed remains, egesta and exuvia were separated and dried. The freshly moulted larvae were weighed and dried. The dried materials were stored for nitrogen analysis.

Food consumption (C), egestion ($F+U$) and production (P) were estimated in terms of dry weight separately for each instar. Dry weight of the infested seed on any day of the experiment was calculated considering the water content of the uninfested control seeds. Faeces egested was estimated by collecting the faeces from each seed separately, on the day of moulting to next instar. Subtracting the dry weight of faeces for the earlier instar(s) from the total dry weight of the faeces on the day of moulting to the next instar FU for each instar was estimated.

Production was calculated by subtracting the dry weight of the larva at the end of each instar from that at the commencement of the instar. Exuviae were pooled, dried and weighed and the mean weight of an exuviae was calculated. For the estimations of consumption, egestion and growth of the 4 larval instars of *C. maculatus* about 1500 infested seeds were dissected. For collecting samples of faeces and insects of different life stages and for estimation of chemical composition, about 1500 seeds were dissected separately at each tested condition.

Samples of food, faeces and larvae of different stages were dried at 80°C to weight constancy. Relating the dry weight of the insect of any chosen stage to its live weight, dry matter content of the stage was estimated. All the weighings were made in a monopan balance (Sauter, West Germany) to an accuracy of 0·01 mg.

Total nitrogen content of the sample (food, faeces and insect) was estimated in a microkjeldahl apparatus following the procedure described by Umbreit *et al* (1972). Each estimation was repeated thrice and an average was taken into account.

The scheme of nitrogen budget followed is the slightly modified IBP formula (Petrusewicz and Macfadyen 1970) represented as $C=P+R+F+U$, where C is the total food consumed, P the growth, R the nitrogen metabolised and $F+U$ the nitrogen loss via faeces; it has been described in detail elsewhere (Muthukrishnan *et al* 1979). The rates are expressed in terms of mg g^{-1} live insect d^{-1}. The total protein, carbohydrate and lipid content of the seeds were estimated following the methods of Lowry *et al* (1951) and Seifter and Dayton (1950) respectively.

2.2 *Statistical procedure*

Regression lines were fitted by the method of least squares. The slopes and intercepts of the regression lines are compared between different components of the food and the parameters. Significant levels were read from the tables given by Zar (1974).

3. Results

Nitrogen content of the food, *C. maculatus* larva and its faeces are provided in table 1. As the larva developing in *D. lab lab* doubled its larval duration and consumed more food, quantity of food nitrogen ingested by it (1·149 mg) was significantly more than that developing in the nitrogen rich *V. unguiculata* (0·528 mg) or

Table 1. Nitrogen content of host seed species, larva of *C. maculatus* and its faeces.

Material	*V. unguiculata* (mg/mg)	*P. radiatus* (mg/mg)	*D. lab lab* (mg/mg)
Host seed species	0·027 ± 0·002	0·025 ± 0·001	0·019 ± 0·003
C. maculatus larva			
I	0·151 ± 0·002	0·165 ± 0·002	0·162 ± 0·003
II	0·151 ± 0·001	0·165 ± 0·001	0·162 ± 0·002
III	0·123 ± 0·004	0·137 ± 0·003	0·235 ± 0·001
IV	0·113 ± 0·002	0·123 ± 0·004	0·225 ± 0·002
V	0·122 ± 0·001	0·132 ± 0·003	0·247 ± 0·003
Faeces (pooled)	0·022 ± 0·001	0·021 ± 0·001	0·016 ± 0·002

Values are expressed in mg dry weight.

P. radiatus (0·558 mg) (table 2). About 75–80% of the dietary nitrogen was lost through faeces and urine corresponding to the low nitrogen content of *D. lab lab*, nitrogen content of the faeces egested by the larva was also less.

Nitrogen assimilated by *C. maculatus* developing in *V. unguiculata*, *P. radiatus* and *D. lab lab* amounted to 0·453, 0·474 and 0·880 mg for the entire larval period at an overall rate of 25·94, 27·07 and 24·48 mg g^{-1} d^{-1} with an overall efficiency of 85·8, 84·9 and 76·6% (table 2). Analysis of variance data show $F(1)2, 24 = 272{\cdot}24$; $F(1)3, 24 = 11{\cdot}79$; $P < 0{\cdot}01$ (table 3). The larva developing in *D. lab lab* accumulated almost twice more nitrogen (0·721 mg) than that developing in *V. unguiculata* (0·378 mg) and *P. radiatus* (0·384 mg) and the rate averaged to 17·3, 14·4 and 12·3 mg g^{-1} d^{-1}. It may be pointed out that contribution by the seed species to the total variance was more than that at the different instars ($F(1)2, 24 = 11{\cdot}3$; $P < 0{\cdot}01$) (table 4).

4. Discussion

Chemical composition of food determines the rate of utilization. Importance of nitrogen in insect-plant interaction has been recently reviewed by McNeill and Southwood (1978) and Mattson (1980). Water has been shown to be an equally important factor in regulating food consumption and growth of insects (Scriber and Feeny 1979). Overall feeding performance of insects is determined by the positive and negative influences of the constituents of the food (Bernays and Simpson 1982).

During the larval period, holometabolous insects accumulate energy in the form of lipid to tide over the non-feeding pupal and adult stages (Gilbert 1964; Waldbauer 1968). Accumulation of nitrogen is essential for egg production during adult stage. Therefore, with increasing nitrogen, fat, energy and water contents the production rate of *C. maculatus* increased (figure 1). Similar results have been reported for the larvae of *Pericallia ricini* by Krishnan (1984). *C. maculatus* which do not feed during the adult stage adopt the following strategies like other lepidopterans (i) shortening of the mortality prone larval duration (Muthukrishnan and Pandian 1984) and (ii) increase in the rate and efficiency of food consumption and conversion during the adult stage (Waldbauer 1968; Pandian 1973).

Assimilation efficiency of *C. maculatus* bears a highly significant positive

Table 2. Effect of seed species on the nitrogen budget of *C. maculatus* developing in *V. unguiculata*, *P. radiatus* and *D. lab lab*.

Parameters	*V. unguiculata*	*P. radiatus*	*D. lab lab*
Duration (D)	16·0	17·5	30·0
NC[a]	0·528	0·558	1·149
NFu[a]	0·075	0·084	0·269
NA[a]	0·453	0·474	0·880
NP[a]	0·378	0·384	0·721
Residual[a]	0·074	0·077	0·159
NCr[b]	30·35	31·65	31·62
NAr[b]	25·94	27·07	24·48
NPr[b]	17·29	14·38	12·31
NAe (%)	85·79	84·95	76·60
N Pe_1 (%)	71·60	68·82	62·75
N Pe_2 (%)	83·44	81·01	81·93

[a]mg individual^{-1}; [b]mg g^{-1} d^{-1}.

Table 3. Analysis of variance for the data on nitrogen assimilation efficiency of *C. maculatus* as a function of life stage and host seed species.

	Host seed		
Life stage	*V. unguiculata*	*P. radiatus*	*D. lab lab*
Instars			
I	85·7 ± 1·1	86·2 ± 2·1	78·2 ± 1·5
II	85·7 ± 1·4	85·5 ± 2·2	77·6 ± 1·2
III	83·9 ± 1·0	83·4 ± 1·7	75·1 ± 1·3
IV	85·1 ± 0·9	85·1 ± 0·9	76·9 ± 0·9

Analysis of variance

Source	*SS*	*df*	*MS*	*F*	*P*
Total	564·62	35	—	—	—
Between host	509·09	2	254·55	272·24	0·01
Between instar	33·08	3	11·03	11·79	0·01
Interaction	3·11	6	0·519	0·555	0·05
Error	22·45	24	0·935	—	—

correlation with nitrogen, energy and water contents of the seed species (figure 1). In a series of publication Pandian and Marian (1985) also reported a positive correlation between assimilation efficiency and food nitrogen for aquatic insects, lepidopterous larvae, polychactes, fishes and reptiles. A low food nitrogen and water enhance feeding rate and suppress the assimilation efficiency of the incumbent (Lawton 1971; Muthukrishnan and Pandian 1984). Hence, while developing in nitrogen and water rich *V. unguiculata*, *C. maculatus* displays a low feeding rate and a high assimilation efficiency. The low efficiency of *C. maculatus* in *D. lab lab* may be due to the presence of saponins which interfere with the process of digestion (Applebaum and Guez 1972; Janzen *et al* 1976).

The positive correlation between net production efficiency and nitrogen may be due to the fact that it has to process more food to acquire nitrogen sufficient

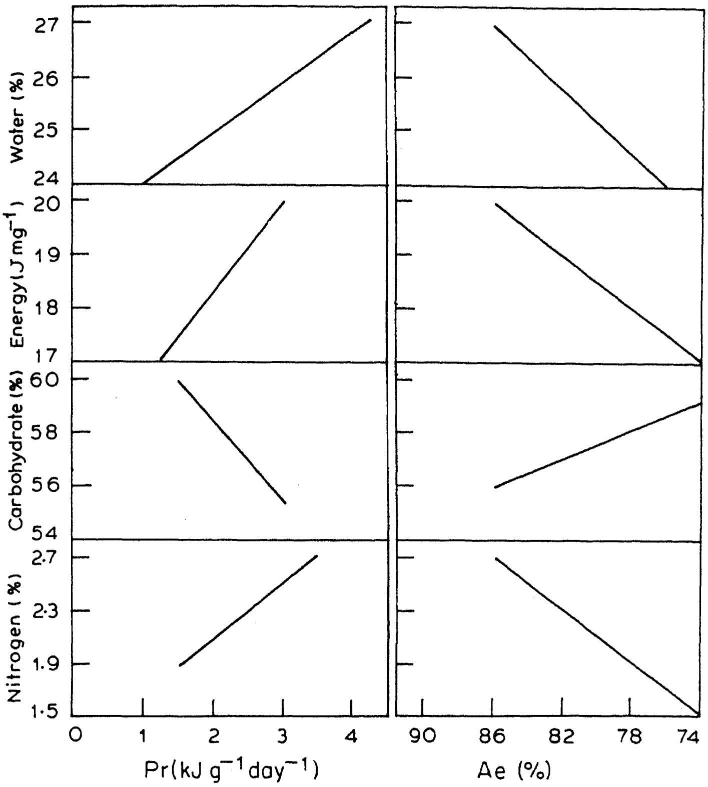

Figure 1. Effects of nitrogen (N), carbohydrate (C), energy (E) and water (W) and contents of grains on rates of production and assimilation in *C. maculatus*.

Pr	$Y = -1{\cdot}850 + 1{\cdot}806$ N;	$r = 0{\cdot}992$
	$Y = -25{\cdot}570 + (-0{\cdot}405)$C;	$r = 0{\cdot}999$
	$Y = -8{\cdot}570 + 0{\cdot}590$ E;	$r = 0{\cdot}998$
	$Y = -6{\cdot}800 + 0{\cdot}363$ W;	$r = 0{\cdot}998$
Ae	$Y = 44{\cdot}07 + 15{\cdot}79$ N;	$r = 0{\cdot}989$
	$Y = 281{\cdot}86 + (-3{\cdot}50)$C;	$r = -0{\cdot}992$
	$Y = -12{\cdot}91 + 5{\cdot}06$ E;	$r = 0{\cdot}976$
	$Y = 4{\cdot}14 + 3{\cdot}04$ W;	$r = 0{\cdot}944$

enough to complete development successfully. The dependence of rate and efficiency of food consumption and utilization on nitrogen content of the seeds is also explained by the significant correlation between developmental rate and nitrogen content. With increasing nitrogen content of the seed, development is accelerated. Larval development in insects involves periodical moulting and synthesis of new cuticle. Availability of nitrogen regulates the process of moulting and formation of cuticle (Gilbert 1964; Wigglesworth 1972). Therefore, development rate of *C. maculatus* feeding in low nitrogen containing *D. lab lab* is slower than that developing in *V. unguiculata* or *P. radiatus*. The gross nitrogen accumulation efficiency (NPe_1) of *C. maculatus* ranges from 62·8% for that developing in *D. lab lab* to 68·8% and 71·6% for those developing in *P. radiatus* and *V. unguiculata*. Food utilization by *C. maculatus* is determined by the nitrogen content of the seed.

Table 4. Analysis of variance for the data on net nitrogen production efficiency (N Pe_2) of *C. maculatus* as function of life stages and host seed species.

	Host seed		
Life stage	*V. unguiculata*	*P. radiatus*	*D. lab lab*
Instars			
I	48·2 ± 1·3	43·1 ± 2·6	26·7 ± 2·2
II	68·6 ± 2·2	56·5 ± 2·1	28·6 ± 1·6
III	53·2 ± 1·9	52·1 ± 1·9	59·4 ± 2·1
IV	86·5 ± 2·4	82·9 ± 1·8	74·9 ± 2·2

Analysis of variance

Source	*SS*	*df*	*MS*	*F*	*P*
Total	12208·69	35	—	—	—
Between host	1823·47	2	911·73	11·30	0·01
Between instar	8494·07	3	2816·35	34·91	0·01
Interaction	1918·43	6	319·73	03·96	0·01
Error	1936·15	24	80·67	—	—

V. unguiculata seed was preferred and more suitable than *P. radiatus* or *D. lab lab* seed for *C. maculatus* because this food source shortened the larval duration, facilitated higher production and enhanced population growth.

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Foraging activity and temperature relations in the ponerine ant *Harpegnathos saltator* Jerdon (Formicidae)

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Abstract. *Harpegnathos saltator* Jerdon constructs nests in the shade of plants and are strictly individual foragers. The nest entrance is barricaded by seeds, seed stalks and faecal pellets of rodents, the function of which is not clear. The diurnal foraging activity is controlled by a combination of surface temperature and light. High midday surface temperature resulted in the bimodal transit activity in a day.

Keywords. Foraging; temperature; light; transit-activity.

1. Introduction

Factors governing the activity of any species are important, since they determine the foraging time available to that species and more so for central place foragers such as ants. Light is the controlling factor for a number of ant species particularly forest dwellers which experience fairly constant temperatures and humidities (Levieux 1975; Levieux and Louis 1975).

The present study was undertaken to determine the factors governing the activity of *Harpegnathos saltator* Jerdon and to establish the temperature range over which activity of these ants occurs. Observations were also made on the nesting behaviour and dietary specialization of this ant. This is also the first ecological observation of a species of genus *Harpegnathos.*

Ants of the genus *Harpegnathos* have few representatives and have limited distributions in the Old World area (Bingham 1903). *H. saltator* is a large (14–18 mm) predatory ant which forages individually and can be compared to the primitive ants of Australia (Taylor 1978). Studies on this genus are not many due to their specific habitat and inconspicuous foraging methods; except for the taxonomic work by Bingham (1903), little is known about these large, jumping, predatory ants.

2. Materials and methods

The present study was undertaken in the tropical scrub forest with eucalyptus plantations (12°57′ N and 77°35′ E) situated in Gandhi Krishi Vignana Kendra Campus of the University of Agricultural Sciences, Bangalore, where 2·5 ha of forest area was thoroughly searched for 20 days to locate all the nests, during cool hours of the day. The nests were located by following a forager returning to the nest with food, and the nests were marked for further observations. To determine the diurnal activity pattern of this ant, hourly visits were made to all the marked nests ($n = 9$).

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The presence or absence of a worker at the entrance for a period of 5 min near the nest was used as an indication of nest activity. Based on this, the per cent nests active at a given time was calculated by using the following formula:

$$\text{Active nests (\%)} = \frac{\text{Number of active nests at a time}\,(t)}{\text{Total number of nests in the study area}} \times 100,$$

where t is the time at which the nest activity was recorded.

The surface temperature near the nests was recorded by using a Diel thermotron, at an hourly interval during the day.

A total of 29·25 h of transit activity was recorded for nest 1 which included the entire foraging observations of this nest for 5 different days during March and April 1987. The transit activity of the ants was recorded from the time of the first worker leaving till the last worker returning to the nest. During this transit activity period, surface temperatures at 15 min intervals were recorded.

The success ratio of foraging was calculated by dividing the number of ants returning with food by the total workers in that foraging session. The success rate per hour basis was calculated by the following formula:

$$\text{Success rate/h} = \frac{\text{Number of workers with food}}{\text{Total number of workers participated in foraging} \times \text{duration of foraging (min)}} \times 100.$$

Differences between the number of workers participating, quality of food collected, success rate and the temperature differences during morning and evening sessions of foraging were analysed using the student's t test.

3. Results

The nests were located by following the workers carrying food. All the nests located were below the plants of varying heights (0·5–2·5 m) having a shade of 2·8–5·5 m^2 during mid-day.

The nest entrance was always barricaded by eucalyptus seeds, seed stalks and faecal pellets of rodents. When the materials were removed or disturbed, additional materials were brought back to the nest entrance during subsequent foraging trips spread over several days.

3.1 *Foraging behaviour*

H. saltator was always found to forage individually. Even a slight disturbance to a forager elicited repeated jumps of 1·5–2 cm and the disturbed ants were found to hide below leaf litter. Nine nests observed for foraging activity at hourly intervals showed two distinct peaks. All the nests were found to be active between 0600–0800 h, while only 66–77% of the nests were found to be active between 0600 and 1900 h. No activity was observed during the rest of the day.

3.2 *Transit activity in nest 1*

The transit activity was never observed during night. The diurnal activity was bimodal during the study period (figure 1). Foraging commenced in the morning

any time from 0502 to 0540 h, while it was observed to take place between 1633 and 1722 h in the afternoon. The mean temperatures at the beginning and during transit activity were 22·72 ± 1·50 and 31·55 ± 1·86°C respectively. Transit activity terminated between 0914 to 0946 (range) and between 1856 to 1943 h. The mean temperature during the termination of foraging was 30·77 ± 1·37 and 27·4 ± 1·47°C respectively for the two sessions. The commencement of foraging coincided with sunrise while the termination coincided with sunset (table 1).

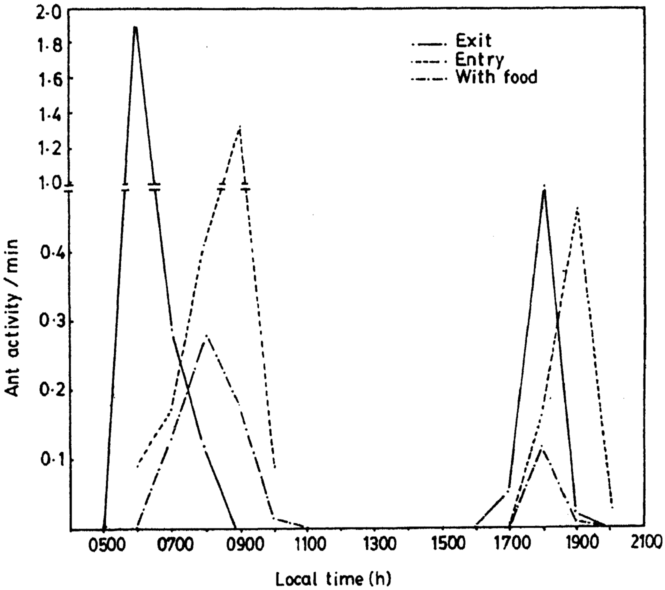

Figure 1. Bimodality in transit activity of *H. saltator* during summer.

Table 1. Transit activity in *H. saltator* during morning and evening foraging.

	Morning $\bar{X} \pm SD$	Evening $\bar{X} \pm SD$	Test statistics
Initiation of foraging	0502–0540 h (range)	1633–1722 h (range)	
Surface temperature (°C)	22·72 ± 1·50	31·55 ± 1·86	
Sunrise	0601–0610 h	—	
Termination of foraging	0914–0946 h (range)	1856–1943 h (range)	
Sunset	—	18·06–18·32 h	
Surface temperature (°C)	30·77 ± 1·37	27·47 ± 1·44	t = 6·67 $P < 0·001$ df = 14
Total duration of foraging (min)	219 ± 7·35	117 ± 25·81	t = 7·6 $P < 0·001$ df = 8
Number of workers participating in foraging (Av. 5 days)	31·8 ± 3·49	10·4 ± 4·67	t = 15·76 $P < 0·001$ df = 8
Success ratio	0·2921	0·1804	
Success rate (%)	7·66	8·66	
Mid-day surface temperature (33·71 ± 3·39°C)			
Morning (24·6 ± 1·21°C)	t = 6·87 $P < 0·001$		
Evening (29·3 ± 1·42°C)	t = 2·88 $P < 0·025$		

The mean foraging duration in the morning was significantly longer ($36{\cdot}5 \pm 0{\cdot}123$ h: $t=7{\cdot}6$ $P<0{\cdot}00$ df=8), than that in the evening ($19{\cdot}5 \pm 0{\cdot}43$) (table 1). Significantly ($t=6{\cdot}67$ $P<0{\cdot}001$ df=8) more number of ants participated in the morning session ($3{\cdot}17 \pm 3{\cdot}49$) compared to the evening session ($10{\cdot}4 \pm 4{\cdot}67$) (table 1). The mean surface temperature during the morning foraging was significantly low ($t=6{\cdot}67$ $P<0{\cdot}001$, df=8) compared to evening hours. However the mean temperatures in the morning and evening of the day were significantly ($t=6{\cdot}87$ $P<0{\cdot}001$ and $2{\cdot}88$ $P<0{\cdot}025$, df=14 and 11 respectively) lower than the mean mid-day temperatures (table 1).

The increase in surface temperature during foraging (morning) resulted in the reduction of transit activity (figure 2).

The success ratio was better in the morning (0·2921) compared to the evening foraging (0·1804). However, the success rate per unit time was lower (7·66%) in the morning compared to evening (8·66%), although the differences were not significant.

Foraging workers collected 36·17 (n=622) and 25·33% (n=75) food in the morning and evening sessions respectively, during the 37 days of observation at nest 1.

The faecal pellets, mostly of rodents, were collected by the unsuccessful foragers on their way back; such materials were rarely carried into the nest.

4. Discussion

Many ponerine ants are known to occur in the thick tree canopy and are forest dwellers. These ants are often found below the shades of undisturbed forest. The reason for the accumulation of seeds, seed stalks and faecal pellets around the nest is

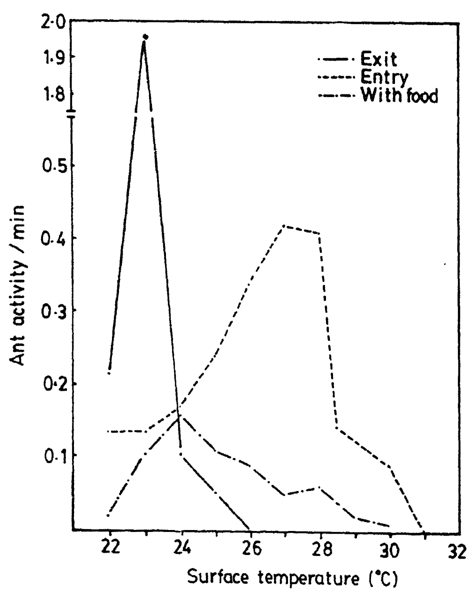

Figure 2. Transit activity in the workers of *H. saltator* decreased with increase in surface temperature.

not clear. *H. saltator* is a strict visual hunter and was never found to use trail pheromones.

Temperature appeared to be a major factor controlling the activity of *H. saltator*. Both the number of nests covered by foraging activity and the transit activity (figure 2) were significantly influenced by this factor. The low rate of increase in the surface temperature and sufficient light available during the morning session were responsible for 100% foraging observed in all the nests while in the evenings, though the temperature was within the foraging range (22–30°C), light restricted the average time for foraging as evidenced by few active nests in the evenings. The mid-day high temperature significantly affected foraging (figure 1) during March and April 1987. Many ant species from different habitats are known to exhibit bimodal pattern of diurnal foraging with mid-day inactivity when surface temperature reaches a certain threshold (DeBruyn and Kruk 1973; Levieux 1975; Whitford *et al* 1976, 1980).

Formica polyctena alternates between bimodal and unimodal activity patterns depending upon surface temperatures (DeBruyn and Kruk 1972). *H. saltator* nests can remain active even during mid-day periods during winter (November–January) and on cloudy days suggesting the existence of a similar phenomenon in this species as in *F. polyctena*. Although the surface temperature was lowest at the beginning of transit activity, it appears that light determined the initiation of foraging as these ants are strict visual hunters. In the evening though the surface temperature was within the foraging range and success rate per unit time was high but the termination of transit activity was observed on account of the non-availability of light due to sunset. Similarly light is the main factor controlling the activity of a number of forest dwelling ants (Levieux 1975; Levieux and Louis 1975). It has been found that in temperate regions low surface temperature results in a total cessation of ants activity (Brian 1965; Sanders 1972), while in *H. saltator* the absence of light has been found to be responsible for total inactivity during night hours.

Food availability and quality can affect the foraging activity (Bernstein 1979; Briese and Macaulay 1980) of ants. *H. saltator* prey was available throughout the day. Adverse microclimatic conditions such as high mid-day surface temperature and lack of light in the evening restricted the available foraging duration in a day. Lack of stored food in the nest was an added evidence of limited foraging duration in this species.

A major proportion of the prey captured were capable of jumping or fast-running, such as crickets and field cockroaches (65%). These ants hunt their prey searching systematically below leaf litter. When they locate the prey, they jump on to it (except for termites) and catch them by their long forceps-like mandibles. The specialization for catching such prey is probably involved in the evolution of jumping behaviour in this species.

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Scanning electron microscopic observations on surface topography of *Isoparorchis hypselobagri* (Billet 1898) Odhner 1927

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Abstract. Scanning electron microscopic observations have been made on the tegument and body pores of the worm *Isoparorchis hypselobagri* (Billet 1898) Odhner 1927, collected from the air bladder of the freshwater fish *Wallago attu* (Schn.). The dorsal and ventral surfaces show characteristic differences under high resolution. Muscles, in hexagonal shape, are arranged on dorsal surface which give a brick floor appearance whereas the ventral surface is occupied by loose, irregular muscles. Sensory papillae are arranged in the excretory pore region around the excretory opening.

Keywords. Scanning electron microscope; *Isoparorchis hypselobagri*; *Wallago attu*.

1. Introduction

Scanning and transmission electron microscopic studies are lacking on Indian trematodes except the work on *Encyclometra colubrimurorum* by Simha and Krishna (1979), *Gastrothylax crumenifer* and *Paramphistomum epiclitum* by Tandon and Maitra (1981), *Phyllodistomum vachius* by Pandey and Tewari (1982) and *Bucephalopsis karvei* by Pandey and Tewari (1984). However, few references on SEM and TEM studies on adult trematodes *Diplostomum phoxini* by Erasmus (1970), *Schistosoma monsoni* by Silk *et al* (1970), *Magalodiscus temperatus* by Nollen and Nadakavukaren (1974), *Fasciola hepatica* by Bennett (1975a, b), *Leucochloridium* sp. and *Urogonimus macrostomum* by Bakke (1976a, b, 1978), *Schistosoma haematobium* by Kuntz *et al* (1976), *Schistosoma japonicum* by Sakamoto and Ischii (1977), *Schistosoma matthei* by Tullock *et al* (1977), *Phyllodistomum conostomum* by Bakke and Lien (1978) and *Clonorchis sinensis* by Fujino *et al* (1979) are available. Currently SEM has been employed by helminthologists to study the surface topography or surface features and other characters of helminth parasites to understand the structural adaptation in different micro-environment. In the present study the observations on surface topography of *Isoparorchis hypselobagri* (Billet 1898) Odhner 1927, an air bladder parasite of freshwater fish *Wallago attu* are reported.

2. Materials and methods

Specimens of *I. hypselobagri* were collected from the air bladder of the freshwater fish *W. attu*, rinsed in 1% saline and fixed in 4% gluteraldehyde prepared in phosphate buffer of pH 6·8. They were dehydrated in graded alcohol series followed by treatment in amyl acetate and then dried by critical point drying using a

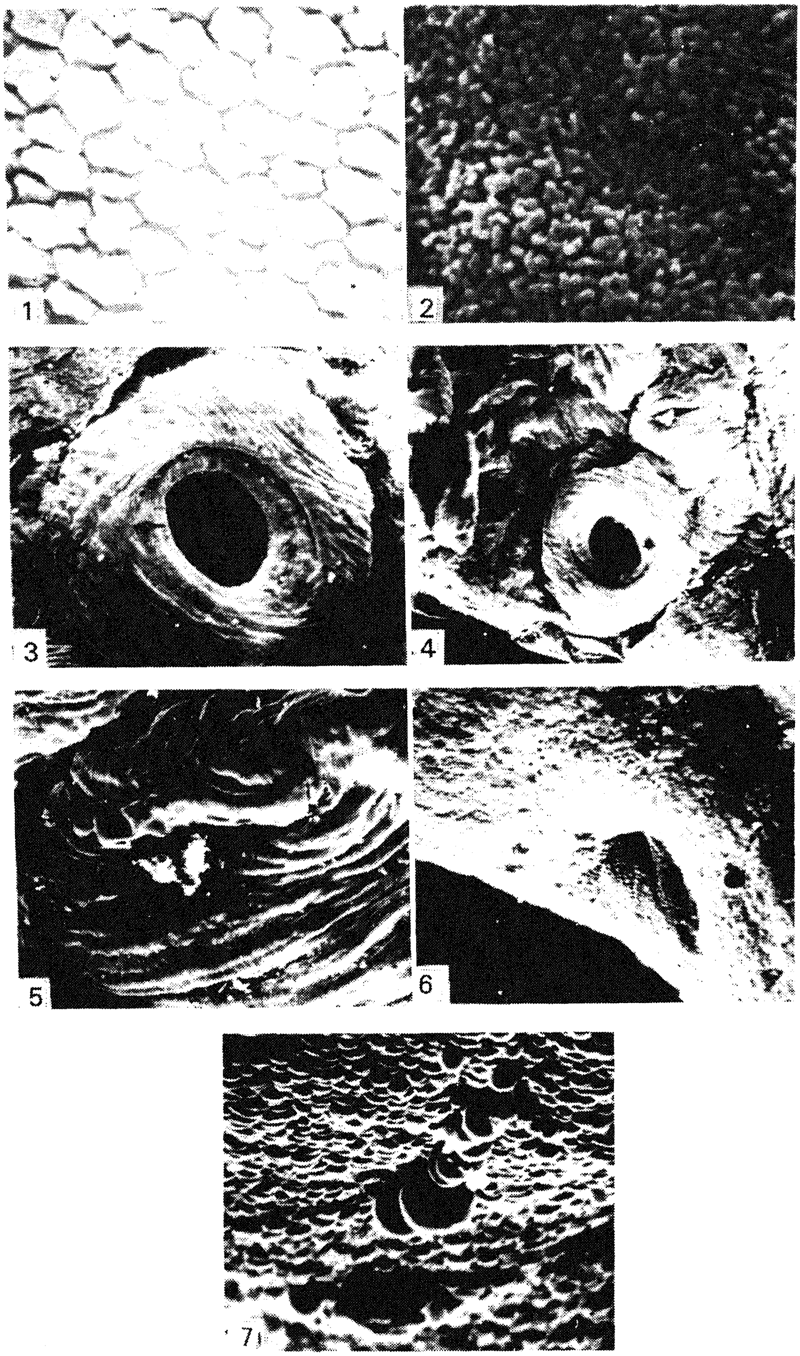
Polaron critical point dryer at 110-PSI. They were mounted on metal stubs followed by coating with gold in Edward 306 vacuum coater. The specimens were observed and scanning electron micrographs taken under a Cambridge steroscan at 60 dl 15 kV at 30° tilt angle.

3. Results

The morphology of *I. hypselobagri* by light microscope has been adequately described by Southwell (1913). The dorsal and ventral surfaces of the tegument show certain characteristic differences under high resolution. No inclusions like spines, cut plates, and pits were observed on the tegument. The dorsal surface (figure 1) is composed of hexagonal muscular structures arranged in linear fashion. It gives a brick floor appearance. However, shape, size and arrangement of the muscles on the ventral body surface (figure 2) are different. This irregular arrangement of the muscles on the ventral surface increases the surface area which is supposed to be helpful in enhancing the attachment capacity with the host body. The tegument around the sucker and genital pore (figures 3–5) is provided with loose circular folds which may also be helpful in increasing the surface area for absorption as well as in the attachment of the worm with the wall of air bladder of the host. The ventral sucker (figure 3) is circular, distinctly made up of two parts viz. central portion containing a rounded opening, and a well developed prominent muscular rim. No sensory papillae, lips, dentition or cut plate or spines are visible inside the opening. The genital pore (figures 4, 5) is postacetabular, located just adjacent to the acetabulum and can be seen as a protruberance, not provided with sensory papillae and depression. The excretory pore (figures 6, 7) is located at hind body margin and gives the appearance of a shallow pit. The excretory pore is not provided with any special muscular arrangements. However, a number of sensory papillae arranged around the excretory pore. These papillae (figure 7) are button-like and of unequal size which is one of the characteristic feature of this parasite.

4. Discussion

The present SEM studies on *I. hypselobagri* reveals various characteristic features on its surface topography. The dorsal and ventral body surfaces show marked differences under the high resolution. The arrangement of the muscles, their shape, and size of dorsal body surface differ from ventral body surface. Moreover, loosely arranged muscles on ventral surface as well as muscular folds around the pores (oral sucker, ventral sucker and genital pore) increase the surface area which probably seems to help the parasite in attachment with host tissue similar to the mode of attachment of *E. colubrimurorum* (Simha and Krishna 1979) and *P. vachius* (Pandey and Tewari 1982). Further, the sensory papillae have not been observed on dorsal and ventral body surface, except in excretory pore regions in *I. hypselobagri*. However, in *U. macrostomum* (Bakke 1978), *Aphalloides timmi* (Bakke 1980) and *P. vachius* (Pandey and Tewari 1982), sensory papillae are confined only around the suckers whereas sensory papillae have been reported on both the surfaces in *F. hepatica* (Bennett 1975b), *Leucochloridium* sp. (Bakke 1976a, b), *Calicophoron calicophorun* (Tandon and Maitra 1982) and *P. vachius* (Pandey and Tewari 1982). Certain variations in the structure of sensory papillae have been observed in



Figures 1–7. **1.** Tegument—SEM of a portion of the dorsal surface of the tegument (× 5000). **2.** SEM of a portion of the ventral surface of the tegument (× 10,000). **3.** SEM of tegument around ventral sucker (× 100). **4.** SEM of a portion of body showing ventral sucker and genital pore (× 50). **5.** SEM of a portion of tegument around the genital pore (× 200). **6.** SEM of the posterior region showing excretory pore (× 100). **7.** SEM of a portion of the tegument around the excretory pore showing papillae (× 5000).

D. phoxini by Erasmus (1970), in *S. mansoni* by Silk *et al* (1970) and Miller *et al* (1972), in *Dilidophora merlangi* by Morris (1973), in *Gorgoderina attenuata* by Nadakavukaren and Nollen (1975), in *F. hepatica* by Bennett (1975b) and in *C. calicophorum* by Tandon and Maitra (1982). Spines, cut plates or similar structures have not been observed anywhere on the body surface of *I. hypselobagri*. However, in *F. hepatica* (Bennett 1975a, b), *Leucochloridium* sp. (Bakke 1976b), *U. macrostomum* (Bakke 1978), *P. conostomum* (Bakke and Lien 1978) and *P. vachius* (Pandey and Tewari 1982) spines are reported on body surfaces except on the suckers which probably help in smooth sealing against the host mucosa. The same function was also presumed by Bakke (1976b) in *Leucochloridium* sp. Differences in structure, distribution and in the number of spines have been observed on the tegument of *P. vachius* (Pandey and Tewari 1982), *U. macrostomum* (Bakke 1978), *Leucochloridium variae micintosh* (Bakke 1982) and *P. conostomum* (Bakke and Lien 1978).

As in *I. hypselobagri* the spines and sensory papillae in the genital pore region are also absent in *B. karvei* (Pandey and Tewari 1984). The button like sensory papillae in the excretory pore region as observed in *I. hypselobagri* are supposed to be helpful in contact communication (tango receptor).

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Isolation of neurosecretory hyperglycemic hormone from the eyestalks of freshwater crab, *Barytelphusa cunicularis*

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Abstract. A procedure is described for the purification of neurosecretory hyperglycemic hormone from the eyestalks of freshwater crab, *Barytelphusa cunicularis*. This procedure involved a combination of gel filtration on Sephadex G-50 and a subsequent preparative polyacrylamide gel electrophoresis which revealed that hyperglycemic hormone moved faster than other proteins.

Keywords. Hyperglycemic hormone; eyestalk; purification; *Barytelphusa cunicularis*.

1. Introduction

Neurosecretory sinus gland complex in the crustacean eyestalk produces, stores and releases a number of hormones which regulate diverse physiological processes. Substantial progress has been made in the complete elucidation of the structures of two eyestalk hormones—red pigment concentrating hormone (Fernlund and Josefsson 1972; Fernlund 1974) and distal retinal pigment light adapting hormone (Fernlund 1971, 1976) in the prawn, *Pandalus borealis*. Hyperglycemic hormone (HGH) is the next most intensely studied eyestalk hormone (Kleinholz and Keller 1973; Kleinholz 1975, 1976). Abramowitz *et al* (1944) first described the diabetogenic action of the eyestalk extract. They found that sinus gland extract of *Uca pugilator* and *Callinectes sapidus* had the effect of increasing blood sugar level in these crabs. Kleinholz and Little (1949) found that such increases in total reducing substances were true hyperglycemias, due to fermentable sugar. HGH has been characterized as a protein of relatively small molecular weight, with an average of 6600 dalton, and several purification steps have been described (Kleinholz *et al* 1967; Keller 1968; Kleinholz and Keller 1973; Skorkowski *et al* 1977). Comparative electrophoretic studies of HGH from the crab, *Cancer magister*; the prawn, *Pandalus borealis* and the crayfish, *Orconectes limosusall* indicate the possibility of species difference in molecular structure of crustacean HGH (Kleinholz and Keller 1973). HGH from 3 different species have different electrophoretical mobilities (Kleinholz and Keller 1973; Keller 1977). Kleinholz (1975) and Keller and Wunderer (1978) have reported the amino acid composition of HGH from *C. magister* and *Carcinus maenas* respectively. Luven *et al* (1982) discussed species or group specificity in biological and immunological studies of crustacean hyperglycemic hormone. Martin *et al* (1984) have isolated and characterized HGH in a terrestrial isopod, *Porcellio dilatatus*. Since most of the work mentioned above is on marine crustaceans, an attempt has been made to isolate HGH from the eyestalks of the freshwater crab, *Barytelphusa cunicularis*.

2. Materials and methods

B. cunicularis were collected from Kham river near Aurangabad and acclimatized to

the laboratory condition. They were fed with pieces of earthworms twice a week and food was withdrawn 24 h before the commencement of the experiment.

2.1 *Extraction*

Sixty eyestalks were removed from mature intermoult crabs, irrespective of their sex. Cuticle and most of the non-nervous tissues were carefully removed. The eyestalks were homogenized thoroughly in 2 ml ammonium acetate buffer (pH 8·5, 50 mM); homogenate was centrifuged for 30 min at 20,000 g in a K-24 refrigerated centrifuge at 4°C. The supernatant was collected and the residue was re-extracted with 1 ml of the acetate buffer and again centrifuged for 30 min. The supernatants were pooled together. This supernatant (2 ml) contained 17 mg proteins (Spector 1978).

2.2 *Gel filtration*

A Sephadex G-50 (superfine) column (1·2 × 64 cm) was used for the separation of HGH, the column was equilibrated with ammonium acetate buffer (pH 8·5, 20 mM). The clear supernatant was applied to the column and elution was carried out with a flow rate of 10 ml/h. Fractions of 1 ml were collected and the protein content and hyperglycemic activity of each fraction were determined.

2.3 *Electrophoresis*

Polyacrylamide gel electrophoresis (PAGE) was carried out in Bronga slab electrophoresis apparatus (Balaji Scientific Service, Madras) using a gel of 7·5% acrylamide at pH 8·9 (Tris-glycine buffer) as described by Davis (1964). The thickness of gel was 15 mm and measured 18 × 18 cm. Samples obtained from the column showing maximum hyperglycemic activity (fraction nos. 43–45) were dialysed against Tris-glycine buffer and were carefully applied with the help of microsyringe into different wells of the gel. Bromophenol blue was used as a tracking dye. Electrophoresis was carried out by applying a current of 12 mA at 8°C until the tracking dye migrated 2–3 cm from the lower end of the gel. A portion of gel containing two wells used for ascertaining the electrophoretic mobility of the applied sample and the remaining major portion used to extract the hyperglycemic activity. The gel was stained with Coomassie brilliant blue R 0·25% in methanol/water/acetic acid (227:227:46, v/v) and destained with sereal changes of methanol: acetic acid: water (10:3:35, v/v). The rest of the gel was cut transversely into 4 segments (3 segments containing 3 band and fourth without any band) based on localization of the bands in the stained gel. Each segment of the gel was separately homogenized in 2 ml of chilled distilled water centrifuged at 4°C for 10 min. The HGH activity was tested in the supernatant.

2.4 *Bioassay*

Crabs of either sex of size 5 × 3 cm were selected. Eyestalks were removed bilaterally and the cut surfaces were cauterized to minimize bleeding. Such destalked animals,

starved for 3 days before use, served as both experimental and control animals. Concentrations of glucose in blood were determined by phenol sulphuric acid method (Dubois *et al* 1956). Blood samples were taken from each of the 6–8 destalked animals of experimental group immediately before and 90 min after the injection of 0·1 ml of test extract. Each animal thus served as its own control. Same procedure was followed with control animals where 0·1 ml of acetate buffer (column chromatography) or glass distilled water (electrophoresis) was injected.

2.5 *Protein determination*

Protein was determined by Coomassie brilliant blue dye procedure (Spector 1978) with bovine serum albumin as a standard protein.

3. Results

The elution of eyestalk extract on Sephadex G-50 column was resolved into 4 different protein peaks (figure 1). Hyperglycemic activity was observed in the II peak (fraction nos 41–47) while peak I (fraction nos 26–40), peak III (fraction nos 48–55) and peak IV (fraction nos 59–68) did not show any HGH activity. It suggests that the first peak corresponds to high molecular weight protein while peaks III and IV contain low molecular weight proteins as compared to HGH.

The eluted sample from Sephadex G-50 with HGH activity subjected to PAGE (pH 8·9) resolved into 3 bands (figure 2). The separate extraction of these bands for HGH assay revealed that HGH activity was associated with the intensely stained and well separated band No. 3 that moved faster than the other two bands (table 1).

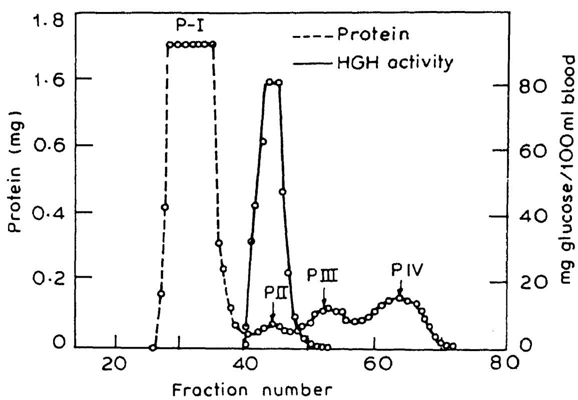

Figure 1. Chromatography of eyestalk extract in ammonium acetate buffer (pH 8·5, 50 mM) from the crab, *Barytelphusa* on Sephadex G-50 (superfine). The column was equilibrated with ammonium acetate buffer (pH 8·5, 20 mM); flow rate 10 ml/h; fraction value 1 ml; total volume 57 ml; void volume 21 ml. The eyestalk extract consisting of 17 mg in 2 ml was applied onto the column. The chromatographic activity of each fraction was tested on 10 animals/fraction and response is depicted in solid line, whereas, protein content is represented in dashed line. P–I, P–II, P–III and P–IV represent various protein peaks.

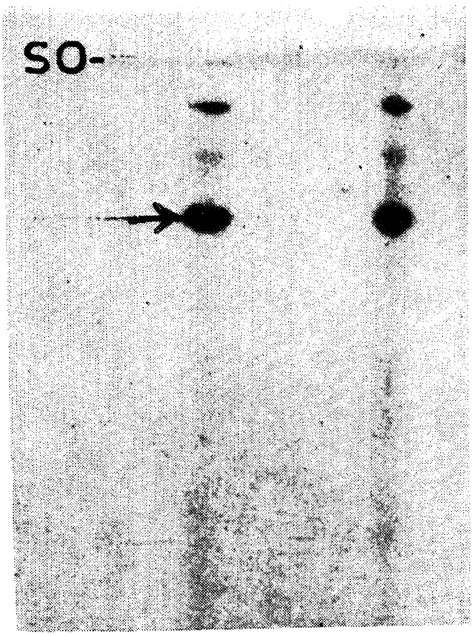

Figure 2. PAGE of the active HGH fraction from Sephadex G-50 conducted according to the method of Davis (1964). Arrow indicates band showing HGH activity. SO, sample origin.

Table 1. Effect of the supernatents of the PAGE segments on the blood sugar level in the destalked crabs, *B. cunicularis* (Dubois *et al* 1956).

Electrophoretic segment No.	Blood sugar level (mg% ± SD)	
	Before injection	After injection
1	7·0 ± 2·9	7·8 ± 8·7^{NS}
2	6·4 ± 3·0	6·9 ± 3·7^{NS}
3	7·5 ± 2·6	22·6 ± 8·2*
4	7·2 ± 2·3	7·8 ± 3·9^{NS}
Glass distilled water control	7·6 ± 1·8	8·6 ± 1·3^{NS}

NS, Not significant. *$P < 0{\cdot}005$.

4. Discussion

The present investigation was undertaken to develop a simplified procedure for the purification of HGH from the eyestalks of the crab, *B. cunicularis* and to confirm its identity with the other crustacean hyperglycemic hormones reported. Purification procedures of HGH from the eyestalks of marine prawn, *P. borealis* (Kleinholz *et al* 1967), shrimp *Crangon crangon* (Skorkowski *et al* 1978) and from the sinus glands of *C. maenas* (Keller and Wunderer 1977) have been reported, whereas, there is no information on the isolation and characterization of HGH in freshwater crustaceans. Initial characterization of HGH from eyestalk extract of *B. cunicularis* has been carried out.

Isolation of HGH from *B. cunicularis* by fractional precipitation with chilled acetone (−10°C) and different concentrations of ammonium sulphate have been tried but did not show any decided advantage because of the poor recovery of the final product. Our approach of the two step isolation procedure by gel filtration and PAGE has been helpful in the elimination of most of the other unassociated proteins as seen in figure 1. Further isolation of the preparation is achieved by electrophoresis and almost homogenous preparation in separate well defined band was obtained even though we have not further tried to purify this. The sinus gland is a much more suitable starting material for isolation of HGH than the whole eyestalk extract. Keller (1977) has shown that HGH is present rather in high quantities in the sinus gland (more than 10%) can be separated from other proteins in PAGE at pH 8·9.

Our efforts in the isolation of sinus glands from the eyestalks were unsuccessful and hence the starting material was contaminated with a number of other eyestalk tissue proteins. We have followed the procedure of Keller and Wunderer (1978) for the isolation of HGH from *B. cunicularis* but the order of technique used by them was reversed. Gel filtration employed first was helpful to characterize a large number of tissue proteins in the eyestalk extract (peaks I, III and IV) which failed to induce hyperglycemia in experimental crabs. HGH activity was associated only with the II protein peak. When PAGE of this II protein peak was carried out, HGH moved faster than other proteins. HGH was found to be associated with the strongest staining band. The presence of HGH activity of *B. cunicularis* in only one peak corroborates with similar activity associated with the HGH isolated from the eyestalks of *P. borealis* (Kleinholz *et al* 1967). Skorkowski *et al* (1977) observed HGH activity in two protein peaks by gel filtration of eyestalk on a Sephadex G-75 column and suggested that two molecular forms of HGH are present in *C. crangon*. The anionic behaviour of *B. cunicularis* and its occurrence in well separated and strongest stained band is in accordance with the reports of Keller (1977) and Skorkowski *et al* (1977).

We have not checked the homogeneity of the final preparation. Hence, further purification, determination of molecular weight, specificity and other properties need further investigation.

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Gill diffusing capacity of a freshwater major carp, *Labeo rohita* (Ham.) in relation to body weight

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Abstract. Emphasis has been placed on providing quantitative data for the water-blood barrier, including diffusion distance, diffusing capacity and overall diffusing capacity based on light microscopic observations. Using values for water-blood barrier obtained from light micrographs, the arithmatic and harmonic mean were found to be 1·88 and 1·32 μm respectively whereas the barrier itself varied from 0·68 to 3·248 μm. Diffusing capacity Dt_1 and overall diffusing capacity Dg for 100 g fish were calculated as 0·5998 and 0·2046 mlO_2/min/mmHg/kg. The diffusing capacity Dt (mlO_2/min/mmHg) showed increasing trend with increase in body weight with a slope of 0·6405 whereas weight specific diffusing capacity Dt_1 (mlO_2/min/mmHg/kg) decreased by a slope of $-0{\cdot}3594$.

Keywords. Diffusing capacity; water-blood barrier; diffusion distance; arithmatic; harmonic mean.

1. Introduction

Diffusing capacity is a phenomenon in respiratory physiology which determines the efficiency of various respiratory membranes for gaseous exchange. The effective gill area and thickness of water-blood barrier are the most important parameters to be considered in determining the diffusing capacity of gases through the respiratory interface.

The thickness of water-blood diffusion barrier in the secondary lamellae of teleosts has been studied by many workers viz. Schultz (1960), Newstead (1967), Hughes and Munshi (1968), Hughes and Wright (1970), Dube and Munshi (1974), Ojha and Munshi (1976) and Roy and Munshi (1987).

The present investigation is an attempt to measure the water-blood barrier and diffusing capacity in relation to body weight in a freshwater major carp, *Labeo rohita* (Ham.).

2. Materials and methods

Live specimens of *L. rohita* were collected from the departmental tank by means of cast net. Small pieces of gills from different weight groups were fixed in Bouin's fixative. The fixed materials were processed and embedded in paraffin and 5–6 μm thick horizontal sections (with reference to the axis of gill filaments) were obtained. The sections were stained by haematoxylin and eosin and their photomicrographs were taken from various levels of gill filaments. The maximum and minimum distance of water-blood pathway were determined directly from photomicrographs. The actual thickness of water-blood barrier was determined by dividing the measured thickness by total magnification. Arithmatic and harmonic means of the thickness of water-blood barrier were determined.

Diffusing capacity was obtained by the rearrangement of modified Fick's equation which is the mathematical representation of the relationship of oxygen uptake ($\dot{V}o_2$) with respiratory surface area (A) and diffusion distance ($\bar{X}h$). The modified Fick's equation (Hughes 1972) may be represented as such:

$$\dot{V}o_2 = \frac{K \cdot A \cdot \Delta PO_2}{t} \tag{1}$$

or

$$\frac{\dot{V}o_2}{\Delta PO_2} = \frac{K \cdot A \cdot}{t} \tag{2}$$

$$Dt = \frac{K \cdot A \cdot}{t} \tag{3}$$

Here $\dot{V}o_2$ = oxygen uptake (mlO_2/min), K = Krough's permeation coefficient (for frog's connective tissue 0·00015 $mlO_2/\mu m$ cm^2/min/mmHg at 20°C), A = gill area (cm^2), ΔPO_2 = partial pressure gradient between water and blood (mmHg), and t = thickness of water-blood pathway (μm).

By applying the values of gill area of *L. rohita* and harmonic mean of water-blood diffusion barrier to equation (3), the diffusing capacity (Dt) was calculated. Regression analysis using logarithmic transformations was made to establish the relationship between the diffusing capacity and body weight. The relationship was expressed by the following allometric equation:

$$Dt = aW^b$$

or

$$\log Dt = \log a + b \cdot \log W$$

(Dt is the diffusing capacity, W body weight, a intercept and b slope value).

The overall diffusing capacity (Dg) for a standard 100 g *L. rohita* was calculated. Hughes (1984) suggested

$$\frac{1}{Dg} = \frac{1}{DW} + \frac{1}{Dm} + \frac{1}{Dt} + \frac{1}{Dp} + \frac{1}{De},$$

where, D = diffusing capacity of, g (gill), W (water), m (mucus), t (tissue), p (plasma) and e (erythrocyte).

As no microridges or microvilli were observed on the secondary lamella under SEM the equation was simplified as

$$\frac{1}{Dg} = \frac{1}{DW} + \frac{1}{Dt} \tag{4}$$

(the main resistent to oxygen diffusion lies through water and tissue)

and $$Dt = \frac{K \cdot A \cdot}{t} \text{ — (1st calculation)},$$

$$DW = \frac{Kw \cdot A}{tw}.$$

Here, Kw = permeation coefficient through water (0·00045 $mlO_2/\mu m$ cm^2/min/mmHg), A = gill area (cm^2) (diffusion area will be the same) and tw = average

thickness of water layer around single face of lamella (1/4th of the interlameller distance: waterpore μm).

3. Results

The water-blood diffusion barrier of the secondary lamella of *L. rohita* comprised an outer layer of epithelium, middle basement membrane and the innermost layer of pillar cell flanges. The total water-blood thickness varied from 0·68–3·248 μm. The arithmetic mean of the same was 1·88 μm whereas the harmonic mean was 1·32 μm.

The gill diffusing capacity (*Dt*) and weight specific diffusing capacity (Dt_1) are summarized in figures 1 and 2.

3.1 *Relationship between body weight and gill diffusing capacity (Dt) ($mlO_2/min/mmHg$)*

The log/log plots of body weight and diffusing capacity gave straight line with their respective slope of 0·6155, 0·6464, 0·6521, 0·6576 and 0·6405 for different gill arches

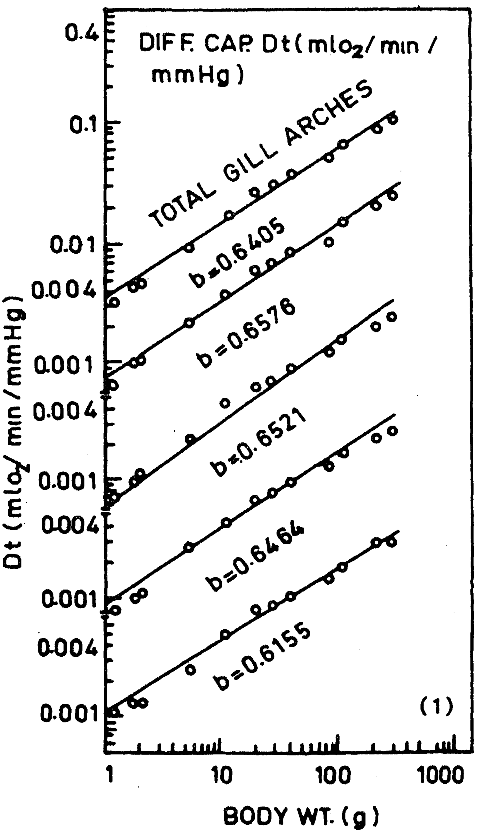

Figure 1. Log/log plots showing the relation between body weight and diffusing capacity (Dt, mlO_2/min/mmHg).

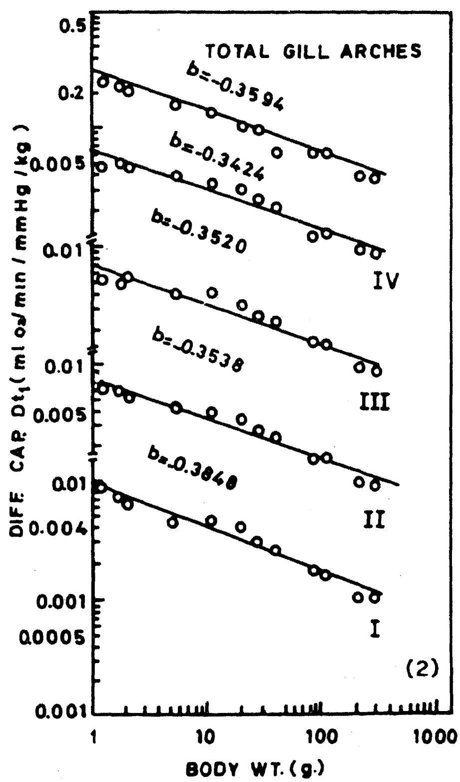

Figure 2. Log/log plots showing the relation between body weight and weight specific diffusing capacity (Dt_1, mlO_2/min/mmHg/kg).

Table 1. Intercept (log *a*), and regression coefficient (*b*) along with their SD and correlation coefficient of the relation to diffusing capacity and body weight in *L. rohita*.

Body weight vs diffusing capacity	Intercept (log *a*)		Regression coefficient (*b*)		Correlation coefficient (*r*)
	Value	SD	Value	SD	
Dt (mlO_2/min/mmHg)					
1st gill arch	0·000986	0·0401	0·6155	0·0131	0·9935
2nd gill arch	0·000768	0·0413	0·6464	0·0135	0·9937
3rd gill arch	0·000717	0·0479	0·6521	0·0157	0·9917
4th gill arch	0·000660	0·0405	0·6576	0·0144	0·9931
Total gill arches	0·00314	0·0410	0·6405	0·0134	0·9937
Dt_1 (mlO_2/min/mmHg/kg)					
1st gill arch	0·9879	0·0400	−0·3848	0·0131	−0·9837
2nd gill arch	0·7691	0·0413	−0·3538	0·0135	−0·9796
3rd gill arch	0·7125	0·0439	−0·3520	0·0144	−0·9796
4th gill arch	0·6608	0·0439	−0·3424	0·0144	−0·9755
Total gill arches	3·1404	0·04106	−0·3594	0·0134	−0·9805

and total gill arches (table 1). The correlation coefficient ($r = 0{\cdot}9937$) for this parameter showed very high correlation.

3.2 *Relationship between body weight and diffusing capacity* (Dt_1) (mlO_2/*min*/*mmHg*/*kg*)

The relationship between the body weight and weight specific diffusing capacity of gill showed high but negative correlation ($r = -0{\cdot}9805$). The bilogarithmic grid always depicted straight lines with respective slope values −0·3848, −0·3538, −0·3520, −0·3424, −0·3594 for different and total gill arches (table 1).

3.3 *Overall diffusing capacity* (*Dg*) (mlO_2/*min*/*mmHg*/*kg*)

The interlamellar distance (waterpore) was measured as $30{\cdot}62 \pm 3{\cdot}2\ \mu m$ so the thickness of water layer around single face of the lamella will be $15{\cdot}31 \pm 1{\cdot}6\ \mu m$. But as the oxygen diffuses from the water layer near both the lamellae and the axis for arriving at the average diffusion distance, the thickness of water layer was again halved to $7{\cdot}65 \pm 0{\cdot}8\ \mu m$. The Dt_1 for 100 g fish was 0·5998 mlO_2/min/mmHg/kg whereas *Dw* was calculated to be 0·3106 mlO_2/min/mmHg/kg. By applying equation (4) *Dg* was calculated to be 0·2046 mlO_2/min/mmHg/kg.

4. Discussion

The metabolic rate of fish depends on its water-blood barrier. In purely water-breathing fishes the barrier is thinner than air-breathing fishes. In *L. rohita* it is thinner (0·68–3·324 μm; harmonic mean 1·32 μm) than the air-breathing fishes such as *Anabas testudineus* (10·00 μm, Hughes *et al* 1973), *Heteropneus fossilis* (3·58 μm, Hughes *et al* 1974), *Clarias batrachus* (7·67 μm, Munshi *et al* 1980), but this value approximates the value for *Mystus* species (1·38 and 2·15 μm, Singh 1979),

Macrognathus acculeatum (1·54, Ojha *et al* 1982), *Glossogobius giuris* (2·70, Singh and Munshi 1985), *Catla catla* (0·885, Kunwar 1984) and *Cirrhinus mrigala* (1·75, Roy and Munshi 1987). The diffusion barrier of tissue is thicker than those reported for active marine fishes like *Katsuwonus pelamis* (0·598), *Euthynus affinis* (0·596, Hughes 1970). From this study it appears that *L. rohita* is a moderately active fish.

The diffusing capacity Dt_1 for a 100 g fish comes to 0·5998 mlO_2/min/mmHg/kg is much higher than many fishes of the same weight group but in comparison to tuna (6·0, Hughes and Gray 1972) it holds lower position.

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Impact of age correlated biochemical changes of host plant on food consumption and utilization efficiency of *Aularches scabiosae* F (Orthoptera: Insecta)

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Abstract. The impact of age correlated biochemical changes in *Tectona grandis* Linn f (Verbenaceae) leaves on the food consumption and utilization efficiency of *Aularches scabiosae* F is provided. The relative preference and maximum utilization of mature leaves when compared to young and senescent leaves are attributed to the changes in the chemical profile particularly protein, nitrogen, carbohydrates, phenols and free fatty acids.

Keywords. Food utilization; leaf age; nutritional changes; *Aularches scabiosa* F.; *Tectona grandis*.

1. Introduction

The significance of chemical composition of plants with regard to host selection between different plant species and its influence on all aspects of insect performance are well known (Chapman and Bernays 1977; Miller and Strickler 1984). The qualitative and quantitative aspects of feeding in acridids (Dadd 1963; Kayed 1983; Ananthakrishnan *et al* 1985) indicate that the biochemical components of the host plants besides the physical factors, decide host suitability. Variations in the nutritional quality between plant species or even within a plant can influence the food preference, growth rate, developmental time and hence the biotic potential of insects (Jermy 1976; Rees 1979; Slansky 1982). In this paper an attempt has been made to study the food consumption and utilization efficiency of *Aularches scabiosae* F in relation to the age correlated nutritional changes in *Tectona grandis* Linn f (Verbenaceae).

2. Materials and methods

Individuals of *A. scabiosae* were collected from the teak forests of western ghats and reared in the laboratory in wooden cages to observe the duration of each instar. Food consumption and utilisation were calculated according to gravimetric method of Waldbauer (1968). Nymphs were weighed after moulting into successive instar. Biochemical estimates of different aged leaves of *T. grandis* were done for total protein (Lowry *et al* 1951), carbohydrates (Dubois *et al* 1956), phenol (Bray and Thorpe 1954), free amino acids (Moore and Stain 1948), nitrogen (Vogel 1963) and lipids (Folch *et al* 1957). Qualitative fatty acids profile of the host leaves of different ages were analysed by Hewlett Packard HPLC system at 230 nm using an Hypersil ODS 5 μm column with water and acetonitrile as solvents at a flow rate of 0·45 ml according to the gradient programme as per Schuster (1985). The retention time and area percentage of free fatty acid methyl esters were recorded.

3. Results

A. scabiosae is a univoltine species feeding on teak leaves. The nymphs pass through 7 instars, each instar period lasting for 15–20 days. Food utilization by different instar stages shows the general trend of very high approximate digestability (AD) and low efficiency of conversion of digested food (ECD) during the early instars which gradually decrease and or increase towards the later instar stages (table 1). Figure 1 shows the biochemical components (%) of food consumed by *A. scabiosae* on different aged leaves of *T. grandis*. This indicates the relative preference of these acridids towards mature leaves with high protein (90 mg/g), lipid (59 mg/g), nitrogen (14·4%) and amino acid (15·79 mg/g) content (table 3). The carbohydrate content was comparatively less (440 mg/g) in mature leaves when compared with young leaf (460 mg/g). Maximum value for water/nitrogen ratio was recorded in mature leaves (6·17). The total phenolic content was the least in mature *T. grandis* leaves.

Table 1. Quantitative food utilization by *A. scabiosae* on *T. grandis*.

Instar	Consumption* (mg)	Excreta* (mg)	Wt. gain* (mg)	CI	GR	AD (%)	ECD (%)	ECI (%)
I	4·851 ± 1·75	2·715 ± 0·86	0·184 ± 0·014	7·081	0·268	44·03	8·614	3·79
II	11·863 ± 1·39	6·377 ± 1·30	0·541 ± 0·275	9·505	0·433	46·24	9·861	4·56
III	19·814 ± 4·44	17·610 ± 1·54	0·989 ± 0·700	4·628	0·231	11·12	44·872	4·99
IV	30·414 ± 3·33	27·535 ± 3·10	1·838 ± 1·217	4·442	0·268	09·46	63·841	6·04
V	58·894 ± 1·02	53·001 ± 0·86	4·560 ± 1·05	10·600	0·433	10·00	77·353	7·74
VI	142·586 ± 3·75	128·327 ± 1·02	9·651 ± 1·13	5·112	0·346	10·15	67·683	6·76
VII	240·278 ± 2·51	185·850 ± 1·60	14·453 ± 2·15	7·624	0·458	22·65	26·55	6·01

*Mean of 5 replicates mg dry wt/day/insect.

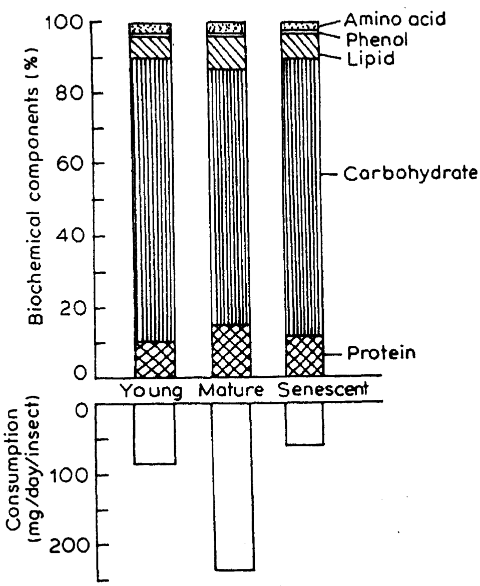

Figure 1. Biochemical components (%) of food consumed by *A. scabiosae* on different aged leaves of *T. grandis*.

Higher food intake in mature and young leaves was also observed (240·278 mg dry wt/day/insect and 81·688 mg dry wt/day/insect respectively). On the contrary, the consumption rate was much reduced in the senescent leaves (71·358 mg dry wt/day/insect) which contain higher proportions of phenols (table 2). Corresponding to feeding there was considerable variation in the weight gain and growth rate by insects when fed on different leaf stages. Individuals feeding on mature leaves showed a higher weight gain (14·45 mg dry wt/day/insect) than on young and senescent leaves. Figure 2 gives the relationship between the various biochemical parameters of different aged leaves and food consumption in *A. scabiosae.*

Age correlated changes in the fatty acid methyl ester of *T. grandis* leaves showed the presence of some early saturated fatty acids, lauric acid and linolenic acid in young and mature leaves. The percentage composition of linolenic acid was comparatively more than lauric acid in young leaves. However, in mature leaves the percentage composition of lauric acid increased to about cent per cent while linolenic acid decreased to only 12%. In senescent leaves lauric acid and linolenic acid were completely absent (figure 3, table 4).

Table 2. Food utilization of *A. scabiosae* (7th instar) on different aged leaves of *T. grandis.*

Leaf age	Consumption* (mg)	Excreta* (mg)	Wt. gain* (mg)	CI	GR	AD (%)	ECD (%)	ECI (%)
Young	81·688 ± 1·54	77·01 ± 1·8	3·57 ± 0·5	5·079	0·221	5·73	75·981	4·36
Mature	240·278 ± 2·51	185·85 ± 1·6	14·45 ± 2·1	7·624	0·458	22·65	26·554	60·15
Senescent	71·358 ± 1·61	67·10 ± 1·5	0·96 ± 0·1	3·217	0·043	6·10	22·010	1·34

*Mean of 5 replicates mg dry wt/day/insect.

Table 3. Chemical composition of *T. grandis* leaves.

Stage	Moisture (%)	Protein (mg/g)	Carbo-hydrates (mg/g)	Lipid (mg/g)	Phenols (mg/g)	Amino acid (mg/g)	Nitrogen (%)	Water/N ratio
Young leaf	59·00	60	460	36	2·83	9·50	9·6	6·14
Mature leaf	88·95	90	440	59	2·37	15·79	14·4	6·17
Senescent leaf	38·22	50	330	30	2·47	9·03	8·0	4·78

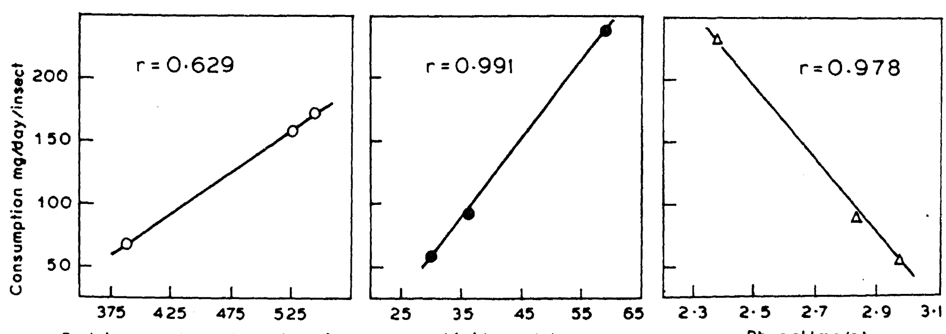

Figure 2. Relationship between various biochemical parameters of different aged leaves of *T. grandis* and food consumption in *A. scabiosae.*

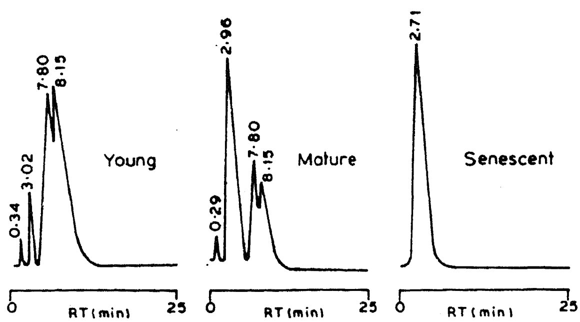

Figure 3. HPLC analysis of fatty acid methyl esters of different aged leaves of *T. grandis*.

Table 4. The retention time and area (%) of the free fatty acid methyl esters of *T. grandis*.

	Young		Mature		Senescent	
	Retention time	Area (%)	Retention time	Area (%)	Retention time	Area (%)
	0·34*	0·118*	0·29*	0·100*	2·71*	100·00*
	3·02*	17·116*	2·96*	77·769*		
Lauric acid	7·80	34·333	7·80	99·446		
Linolenic acid	8·15	48·443	8·15	12·685		

*Earlier fatty acids.

Food utilization efficiency in *A. scabiosae* shows variation in relation to changes in the nutrient contents of the leaves. The utilization efficiencies shows higher values in mature leaves. Hówever, in senescent and young leaves the efficiencies were so adjusted by the insect to compensate for the reduced feeding (table 2).

4. Discussion

The seasonal variation in food quality found in many plants (McNeill and Southwood 1978; Prestidge and McNeill 1982) particularly the protein content is often limiting for insect development (Mattson 1980). The amount of food consumed and consequent growth occurring during the final instar comprise over 75% of the totals for the whole larval stage (Waldbauer 1968) and tend to be a representative of those calculated for the entire larval stage (Scriber and Slansky 1981). In the present study differences in the total quantity of ingested food by the 7th instar *A. scabiosae* from young, mature and senescent leaves of *T. grandis* might be attributed to the difference in the nutritive quality in relation to the age of the leaves, since the selection of food by acridids generally have a greater chemical basis (Bernays and Chapman 1970). In acridids water content of the leaves undoubtedly affect food consumption. The moisture content of *T. grandis* leaves declines from 88% in mature leaves to 38% in senescent leaves. It is known that acridids take larger meals on lush grass with high water content (Chapman and Bernays 1977). Similar to *A. scabiosae*, *Nomadacris* also exhibited a preference for grasses with high water content (Chapman 1957). However, Bernays and Chapman (1970) found no

difference in the acceptability of grass with different moisture content to *Chorthippus parallelus.*

In addition to the reduction in water content there tends to be a reduction in the protein especially the nitrogen content and an increase in the secondary substances during senescing (Feeny 1976; Scriber and Slansky 1981). The final instar period which is a period of somatic growth is characterised by intense feeding activity. During this period considerably more protein is ingested. In desert locust omission of bran with high protein content slowed down the rate of somatic growth (Hill *et al* 1968). The consumption and the weight gain by *A. scabiosae* increased when fed with mature leaves, containing about 90 mg/g of protein and 14·4% of nitrogen. The growth rate of insects are generally more closely correlated with nitrogen in the leaves (Scriber and Feeny 1979). In this study, consumption and weight gain by *A. scabiosae* were significantly low in senescent leaves due to lesser concentrations of nitrogen and protein. Moreover the higher leaf water/nitrogen ratio in mature *T. grandis* leaves significantly increased the approximate digestability in *A. scabiosae* (Manuwota 1984; Slansky and Feeny 1977) (table 3). Higher growth rate and higher weight gain of *A. scabiosae* per day on mature leaves closely paralleled with the total leaf nitrogen, because of this strong correlation of approximate digestability with leaf nitrogen. Hence, the decline in the nitrogen and moisture content of senescent leaves are found to reduce the suitability of leaves as food for *A. scabiosae* and also the digestability.

Except for cholesterol and some amounts of fatty acids, acridids have no major requirements for lipids. An assessment of fatty acid methyl ester profiles of *T. grandis* leaves showed the presence of some early saturated fatty acids, lauric acid and linolenic acid in mature and young leaves, which are important among dietary fatty acids. The ability to synthesize linolenic acid is generally lacking in insects and hence should be available in the diet (Gilbert 1967; Fast 1970; Downer 1978). Absence of linolenic acid in the diet caused the failure of pupal/adult ecdysis in several lepidopterans, like *Ephestia, Spodoptera littoralis* (Vanderzant *et al* 1957; Levinson and Navon 1969). Acridids show fatty acid deficiency by the emergence of deformed adults at the final moult preceded by retarded nymphal growth (Dadd 1963; Nayar 1964). *Locusta migratoria* was found to be more sensitive to the absence of fatty acid in the diet than *Schistocerca gregaria* (Dadd 1960). Lower consumption by *A. scabiosae* on senescent leaves containing none of these two fatty acids but only one of the earlier saturated fatty acids could be the cause for poor weight gain and low growth rate.

In *L. migratoria*, Worm and Beenakkers (1980) reported maximum assimilation of carbohydrate during somatic growth which was accounted as a source for carbon to build up chitin to be deposited in the cuticle. In mature leaves of *T. grandis* the availability of carbohydrates was low when compared to young leaves. The feeding preference of *A. scabiosae* attest the results obtained by Cook (1977) where very high concentrations of sugars limit the amount of food consumed. In the present investigation the relationship between the food consumed and the additive effect of protein carbohydrate and amino acids shows a positive correlation (figure 2). But the total phenolic content of different age groups of *T. grandis* leaves appear to interfere with the food consumption. High phenol content in senescent leaves reduced the food consumption by *A. scabiosae* as evidenced in other acridids like *Oxya nitidula, Atractomorpha crenulata* and *Aiolopus thalassinus* (Sanjayan and

Ananthakrishnan 1987). On the contrary plant phenols which are regarded as deterrent to insects has been shown to improve growth and survival of the acridid *Anacridium melanorhodom* (Bernays and Woodhead 1982).

Hence it appears very evident that the chemical composition of *T. grandis* is of significance in consumption and utilization of food by *A. scabiosae.* Moreover the close phenological synchronization of *A. scabiosae* nymphs and adults with the appearance of lush green leaves of *T. grandis* by late January and the completion of growth and reproduction before the food quality declined (from late November to January when leaf shedding occurs) also appears noteworthy.

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Proc. Indian Acad. Sci. (Anim. Sci.), Vol. 98, No. 6, November 1989, pp. 399–404.

Effect of rutin on the neonate sensitivity, dietary utilization and mid-gut carboxylesterase activity of *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae)

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Abstract. Rutin (quercitin-3-β-D-rutinoside) a widely occurring plant glycoside affects neonate survival and inhibits early larval growth of the tobacco leaf eating caterpillar, *Spodoptera litura* (F) when added to an artificial diet for this insect. Dietary concentration of rutin up to 1% wet weight had no adverse effect on the weight gain, nutritional indices (approximate digestibility, efficiency of conversion of assimilated and ingested food) when fed to early V instar larvae over a period of 48 h. Rutin was excreted unchanged and the overall amount excreted was $\sim$50%. Neonate larvae reared on control diet till early V instar and subsequently fed on high doses (0·1 to 1%) of rutin did not exhibit any increase in the mid-gut carboxylesterase activity. Similar rearing on sublethal doses (0·01 to 0·1%) of rutin, followed by feeding of a high dose (1%), resulted in significant increase in the mid-gut carboxylesterase activity of V instar larvae.

Keywords. Rutin; *Spodoptera litura*; neonate sensitivity; nutritional indices; carboxylesterase; induction.

1. Introduction

The hypothesis that plant secondary compounds may serve a protective function against herbivory appears to be well accepted (Feeny 1976; Rhoades and Cates 1976) albeit not always as well documented (Reese 1979). Natural food plants contain not only nutrients but also allelochemicals. The latter compounds presumably serve as a defence system to deter herbivores. This necessitates that these insect species which adopt a specific plant as food sources have to neutralize the toxic effects of its allelochemicals.

Rutin, a widely occurring plant glycoside, has been shown to have insect growth inhibiting effects on a number of agricultural pests (Shaver and Lukefahr 1969; Elligar *et al* 1981; Isman and Duffey 1982). Since rutin is considered as a ubiquitous flavonoid amongst terrestrial plants (Sondheimer 1964; Harborne 1979), from a purely ecological perspective, we might expect that *Spodoptera litura*, a generalist herbivore would be able to detoxify or in some manner avoid toxicity from such ubiquitous secondary compound. Certain physiological and biochemical responses of *S. litura* to rutin have been examined to elucidate these aspects.

2. Materials and methods

Larvae of *S. litura* were maintained on artificial diet at 28 ± 2°C and a 12:12 L:D photoperiod. Diets were prepared after Nagarkatti and Prakash (1974).

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Rutin (Sigma Chemical Co., St. Louis, Missouri, USA), was admixed into the dry portion of the diet prior to preparation owing to its limited solubility in water.

Neonate sensitivity to rutin on survival and growth were studied by placing 20–25 neonate larvae individually on about 1 g wet weight of diet with or without rutin and allowing the larvae to feed for 24 h in case of survival experiments and for 3 days, till all the larvae in the control lots moulted into the II instar, for growth experiments. All experiments were replicated 5 times.

To investigate the possible effects of rutin on dietary utilization by *S. litura*, cohorts of larvae were first reared on control diet. Freshly moulted early V instar larvae were transferred to pre weighed portion of diets containing different concentrations of rutin (0·1 to 1%). After 48 h, the larvae, frass and remaining diet each were weighed in a Sartorius MP 2024 balance and kept in an oven at 60°C for 48 h for obtaining dry weights. Nutritional indices, viz. weight gain, approximate digestibility (AD), efficiency of conversion of assimilated food (ECD) and efficiency of conversion of ingested food (ECI) were calculated on the basis of dry weights as outlined by Waldbauer (1968). The frass obtained was further subjected for estimating rutin spectrophotometrically at λ 357 (Porter *et al* 1947), using a Shimadzu UV-260 spectrophotometer. All experiments were replicated 5–7 times.

To study the mid-gut carboxylesterase (CE) (EC 3·1·1·1) activity groups of 10 mid-guts were dissected out from the larvae, which were fed on rutin treated diets of different concentrations for 48 h, in 1·15% KCl and their gut contents removed. Tissue were drained of KCl solution and rinsed with 0·04 M phosphate buffer (pH 7). Rinsed tissues were then homogenized in 1 ml of ice-cold phosphate buffer per 3 mid-guts in a motor driven tissue grinder/homogenizer. Homogenate thus obtained was centrifuged at 10,000 *g* for 10 min at 4°C. The supernatant was used directly as enzyme source. Mid-gut CE activity was determined by van Asperen's (1962) method, using 1-naphthyl acetate as the substrate. Protein estimation was done by Lowry's method (1951).

3. Results and discussion

3.1 *Neonate sensitivity experiments*

Rutin generates linear dose-response curves for *S. litura* with respect to mortality of the neonate larvae and its ability to moult into the II instar (figures 1, 2). In the present study, we have shown that rutin is toxic by two criteria, survival of neonate larvae and its ability to moult into the next instar, which is based on symptomology alone.

The observation that neonate larvae of *S. litura* are sensitive to rutin whereas V instar are not and the fact that there is an increase in the mid-gut CE activity of V instar whose early instars had been exposed to sub-lethal doses of rutin, suggests that neonate sensitivity/toxicity may be due to lack of detoxifying enzymes in the gut of the freshly hatched larvae as has been found in case of certain herbivores (Gould 1984; Cohen 1983). Several other modes-of-action have been suggested by Somogyi and Bonicke (1969), Davies *et al* (1978), Hoover *et al* (1977) and Wagner (1979). Perhaps the modes-of-action of rutin, both behavioural and physiological, change during the larval life of the insect, making it highly improbable to argue to a single mode-of-action.

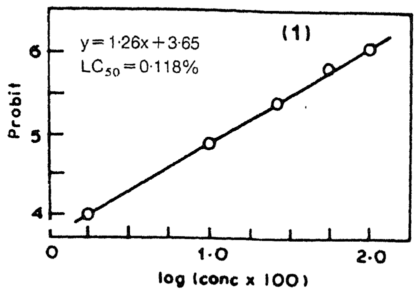

Figure 1. Mortality of neonate larvae of *S. litura* on rutin treated diet.

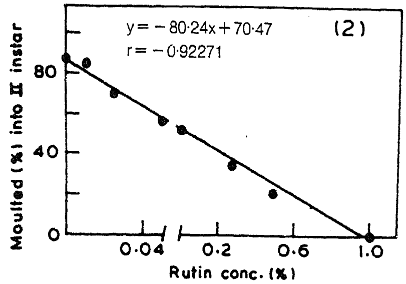

Figure 2. Moulting of neonate larvae of *S. litura* to II instar on rutin treated diet.

3.2 *Feeding and nutrition experiments*

Rutin even at dietary concentration of 1% (far exceeding that found in plants) did not affect the weight gain, nor did it reduce digestibility or affected dietary utilization of food by the early V instar larvae (table 1).

Rutin was excreted unchanged and the overall amount excreted was approximately 50% (table 2). This suggests that one of the ways of avoiding toxicosis from rutin by the older larvae of *S. litura* is to eliminate the allelochemic unchanged through excreta. There are a few studies which indicate that plant compounds excreted in the frass of generalist insects are unchanged from their original structure (Self *et al* 1964; Isman and Duffey 1983; Duffey 1980).

3.3 *CE activity experiments*

Rutin fed for 48 h at different concentrations (0·1 to 1%) to the early V instar larvae had no significant effect on the mid-gut CE of *S. litura* (table 3). Alternately, neonate larvae reared on sub-lethal doses throughout the experiment and sacrificed when 48 h old in the V instar stage show significant increase in the mid-gut CE activity compared to the control ($F=3{\cdot}9$, $P<0{\cdot}05$) (table 4). Also neonate larvae reared on sublethal doses (0·01 to 0·1%) when transferred at early V instar stage to 1% rutin treated diet exhibit significant increase in the mid-gut CE activity compared to the controls ($F=12{\cdot}8$, $P<0{\cdot}05$) (table 5). Our findings with the older larvae agree with Yu (1985) wherein 0·2% quercitin has no significant effect on the mid-gut esterases of *S. frugiperda*. It has recently been found that in phytophagous insects the detoxication enzymes can all be induced by plant allelochemicals (Brattsten *et al* 1979; Yu 1986). Such induction leading to an increase in CE activity on exposure to sublethal doses of rutin have important implications in the pest control schedules, since increase in CE activity can result in decreased toxicity to insecticides containing ester linkage.

4. Conclusion

Neonate larvae are far more sensitive to rutin than older larvae. There are several possible mechanisms through which this compound could interfere with larval growth. Changes in the sensitivity to allelochemics may revolve around the

Table 1. Relationship between dietary concentrations of rutin and weight gain (mg), AD, ECD, and ECI.

Concentration of rutin in diet (%)	Weight gain (mg) $\bar{X} \pm SE$	AD $\bar{X} \pm SE$	ECD $\bar{X} \pm SE$	ECI $\bar{X} \pm SE$
0·00	82·12 ± 3·22	47·22 ± 1·92	69·66 ± 2·74	27·89 ± 0·51
0·1	76·01 ± 3·62	39·68 ± 1·84	70·98 ± 1·68	27·92 ± 0·66
0·25	75·84 ± 4·05	39·86 ± 0·78	69·51 ± 1·38	27·69 ± 0·64
0·5	75·22 ± 3·06	40·95 ± 2·01	61·09 ± 2·60	24·96 ± 1·11
1·0	76·60 ± 3·15	38·91 ± 1·14	67·01 ± 1·73	26·07 ± 0·99

All replicates were pooled for data analysis by ANOVA.
Values are not significant from the control ($P > 0·05$).

Table 2. Per cent rutin elimination in the excreta of *S. litura*.

Concentration of rutin in diet (%)	Rutin elimination (%) (Mean ± SD)
0·1	43·4 ± 0·06
0·25	47·75 ± 1·66
0·5	52·86 ± 9·38
1·0	55·37 ± 7·97

Table 3. Effect of rutin on the mid-gut CE activity of early V instar larvae of *S. litura*[a].

Rutin concentration in diet (%)	Specific activity[b] (nmol/min/mg protein 1-naphthol)	Control (%)
0·00	308 ± 26·95	100
0·1	267·5 ± 27·3	87
0·25	326 ± 19·67	105
0·5	348 ± 26·80	113
1·0	357 ± 43·54	116

[a]Newly moulted V instar larvae (previously fed on meridic/control diet) were fed on meridic diets containing rutin at different concentrations for 2 days (48 h) before enzyme assay.
[b]Mean ± SE of 4 replicates.
All replicates were pooled for data analysis by ANOVA.
Value is not significant ($P > 0·05$) from the control.

induction of detoxification system. Although there are reports on the effect of plant allelochemicals on mid-gut MFO's and glutathion-S-transferases, little is known about the effects of host plants and allelochemicals on esterase activity in insects. Future investigations of insect-allelochemic interactions should include consideration of herbivore age-specific effects, and should stress more on integrative use of toxicology to document the effect of plant-secondary compounds on insects.

Table 4. Effect of rutin on the mid-gut CE activity in the V instar 48 h stage *S. litura* reared on sub lethal doses of rutin throughout the experiment.

Rutin conc. (%) in diet	Sp. activity[a] (nmol 1-naphthol/min/ mg protein)	Control (%)
0·00	328 ± 18·95	100
0·01	399 ± 5·65	121·65
0·025	389 ± 29·2	118·60
0·05	405 ± 7·75	123·48
0·1	422 ± 6·05	128·66

[a]Mean ± SE of 4 replicates.
Replicates were pooled for data analysis by ANOVA.
$F = 3{\cdot}9$; $P < 0{\cdot}05$.

Table 5. Effect of 1% rutin on the mid-gut CE activity of V instar larvae of *S. litura*[a].

Rutin concentration in diet (%)	Specific activity[b] (nmol/min/mg protein 1-naphthol)	Control (%)
0·00	362 ± 2·55	100
0·01 to 1·0	480 ± 23·4	133
0·025 to 1·0	407 ± 3·45	112
0·05 to 1·0	463 ± 12·4	128
0·1 to 1·0	466 ± 23·4	129

[a]Newly moulted V instar larvae from the respective sub lethal doses of rutin were fed on diet containing 1% rutin for 48 h before enzyme assay.
[b]Mean ± SE of 4 replicates.
Replicates were pooled for data analysis by ANOVA.
$F = 12{\cdot}83$; $P < 0{\cdot}05$.

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Persistent occurrence of spermatogenesis in the adult testes of *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae)

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Abstract. The histology and ultrastructure of the adult testis of *Spodoptera litura* are studied using light microscopy and electron microscopy. Maturing spermatocytes, spermatids and their conditions within the testes are described. The continued occurrence of spermatogenesis in the adult testes has been traced and reported.

Keywords. Follicle; sertoli cells; spermatid; spermatocyte; spermatogonia; testis.

1. Introduction

The morphology of male reproductive tracts of many Lepidoptera has been described while the ultrastructure of the same is limited. Testicular development has been studied in a number of Lepidoptera (Snodgrass 1935: Chaudhury and Raun 1966; Holt and North 1970; Retnakaran 1970; Chase and Gilliland 1972; Sạlama 1976; Brits 1979a, b; Lai–Fook 1982). Morphology and histology have been described in a number of species (Musgrave 1937; Callahan and Chapin 1960; Callahan and Casio 1963; Thibout 1971; Leclercg–Smerkens 1974; Santorini and Vassilaina–Alexopoulou 1976; Amaldoss 1988). In this paper a study of adult testes has been taken up by using light microscopy (LM) and electron microscopy (EM) techniques and the occurrence of spermatogenesis has been traced and reported.

2. Materials and methods

The experimental moths were reared at 25–28°C in an artificial medium (Biomix 9798, New Jersey, USA) and were kept in a 12:12 photoperiod in the growth chamber of Long Light Company, Taiwan. Relative humidity was $60 \pm 5\%$. The adult moths were fed with 10% honey solution. In this experiment either 2-day old virgin males or 4-day old mated males were used.

2.1 *LM sections*

Testes from the male reproductive tract were dissected open in the Weevers (1966) Lepidopteran saline. Testes of adult virgin males and mated males are fixed in Zenker solution for 3 h and then dehydrated in alcoholic series and embedded in Paraplast. Five μm sections were cut and the sections were stained in Meyers hematoxylin and counter stained in eosin. Then photographs were taken in a phase contrast microscope.

2.2 EM sections

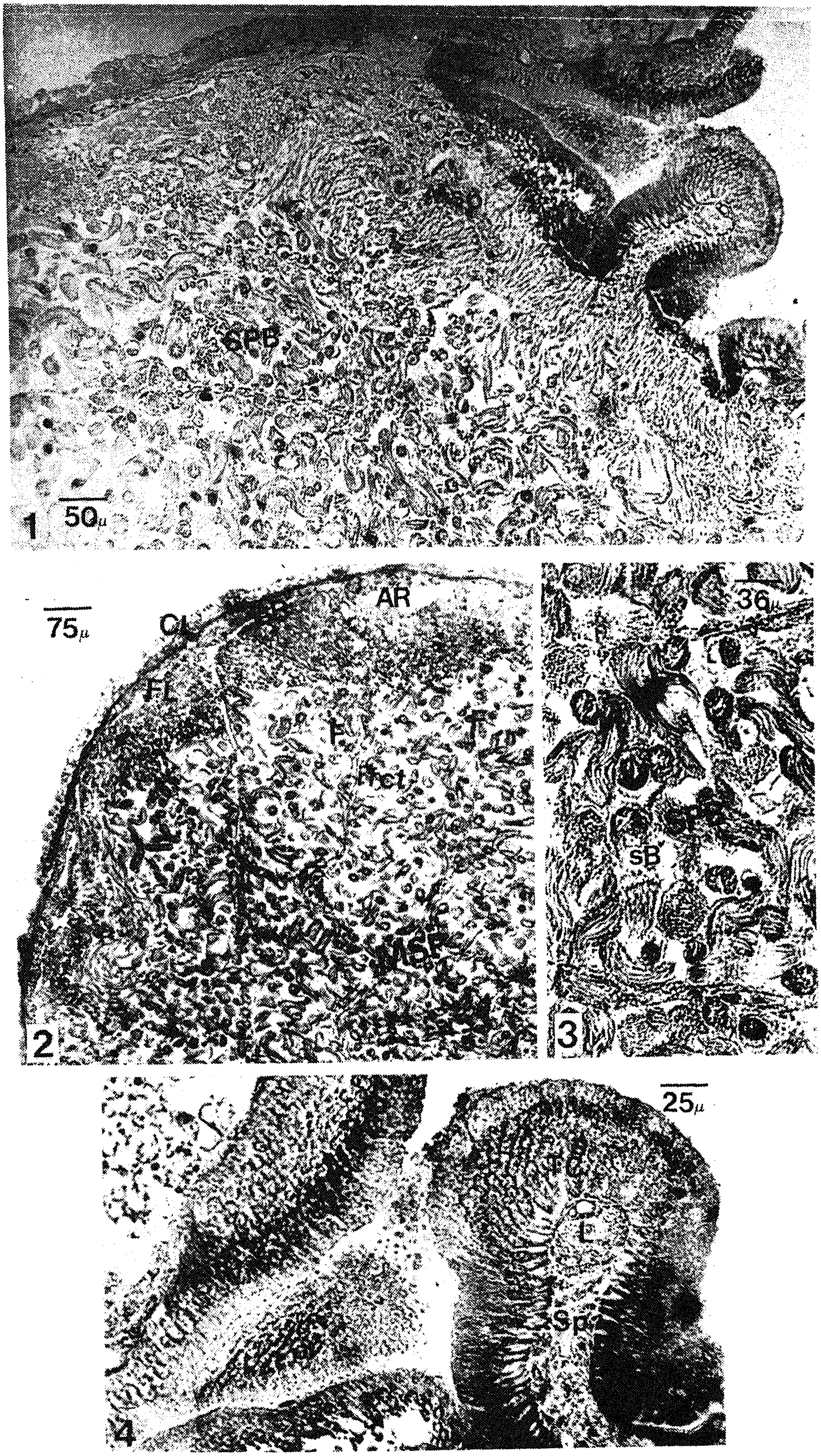
The tissues were fixed in 2·5% glutaraldehyde in pH 7·2, 0·1 M of phosphate buffer at 4°C and then post fixed in 1% osmium tetroxide in the same condition. After dehydration in acetone series, tissues are embedded in Spur EPON 812. Thin sections were cut and then stained with uranyl acetate and lead citrate and viewed under JEM 100S at 45 KV.

3. Results

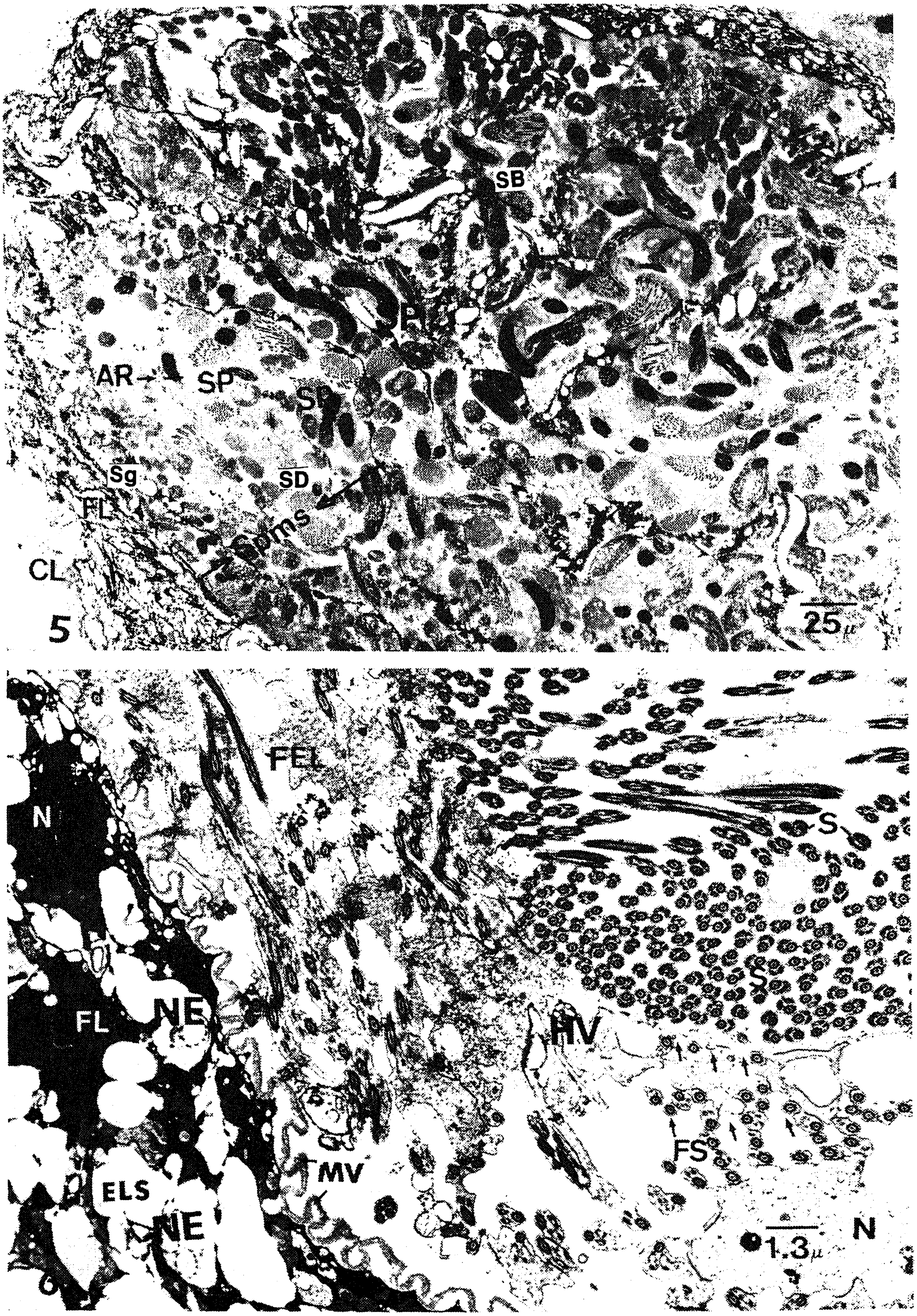
Deep yellow colored testes of *Spodoptera litura* are paired and is easily distinguishable in the abdomen upon dissection. But they appear as a single organ because of being closely united. The testes lie on the dorsal side between the 4th and 7th segments attached to the dorsal epidermis by means of tracheae and basement membrane like substance. The testes are 0·1 mm long and 0·12 mm broad. The testes are enclosed in a scrotum. This external wall, which is 2–4 μm thick can be carefully removed by means of fine foreceps. There appears to be a large number of tracheae occurring on the surface of the scrotum wall. The outer wall of the testes appear colourless and structureless, while the layer beneath the wall of scrotum appears deep yellow and is 2–3 μm thick. Nerve endings penetrate deep into this layer also. The inner layer runs beyond into the interior part of testes dividing them into 8 incomplete compartments called follicles. The layer that divides two follicles is called interfollicular connective tissue. Under LM and thick section of EM, it is possible to notice the apical region of the testes with spermatogonia followed by spermatocytes, spermatids, spermatozoa and sperm bundles towards the opening of the testicular calyces. Immature spermatocyte at the periphery of testes to mature spermatozoa in large numbers rushing out into the testicular calyces. Each follicle contains many cysts which have various stages of spermatogenesis and spermiogenesis. The testes are on the ventral side extended as paired inflated structures as testicular calyces and they cross each other at their point of entry. Testicular calyces are continuous with non celluar layers of testes. They are surrounded by a 3·1 μm thick basement membrane. The epithelium of testicular calyces is secretory in nature and

Figures 1–4. **1.** A part of testis on the testicular calyx (TC) side of *S. litura* showing distinct areas (i) stationary spermatozoa (SP) in bundles, (ii) sperm bundles (SB) and individual spermatozoa appear to leave the testes into testicular calyx and (iii) mature spermatozoa (MSP) testicular calyx and its lumen with spermatozoa. **2.** Apical region (AR) of the testis showing the capsule layer (CL), follicular layer (FL), follicles (F) and interfollicular connective tissue (IFCT). Also it is evident to notice the immature and mature spermatozoa (MSP) from apical region to the center. **3.** High magnification of sperm bundles (SB). **4.** Testicular calyx showing its epithelium, lumen (L) and its secretion.

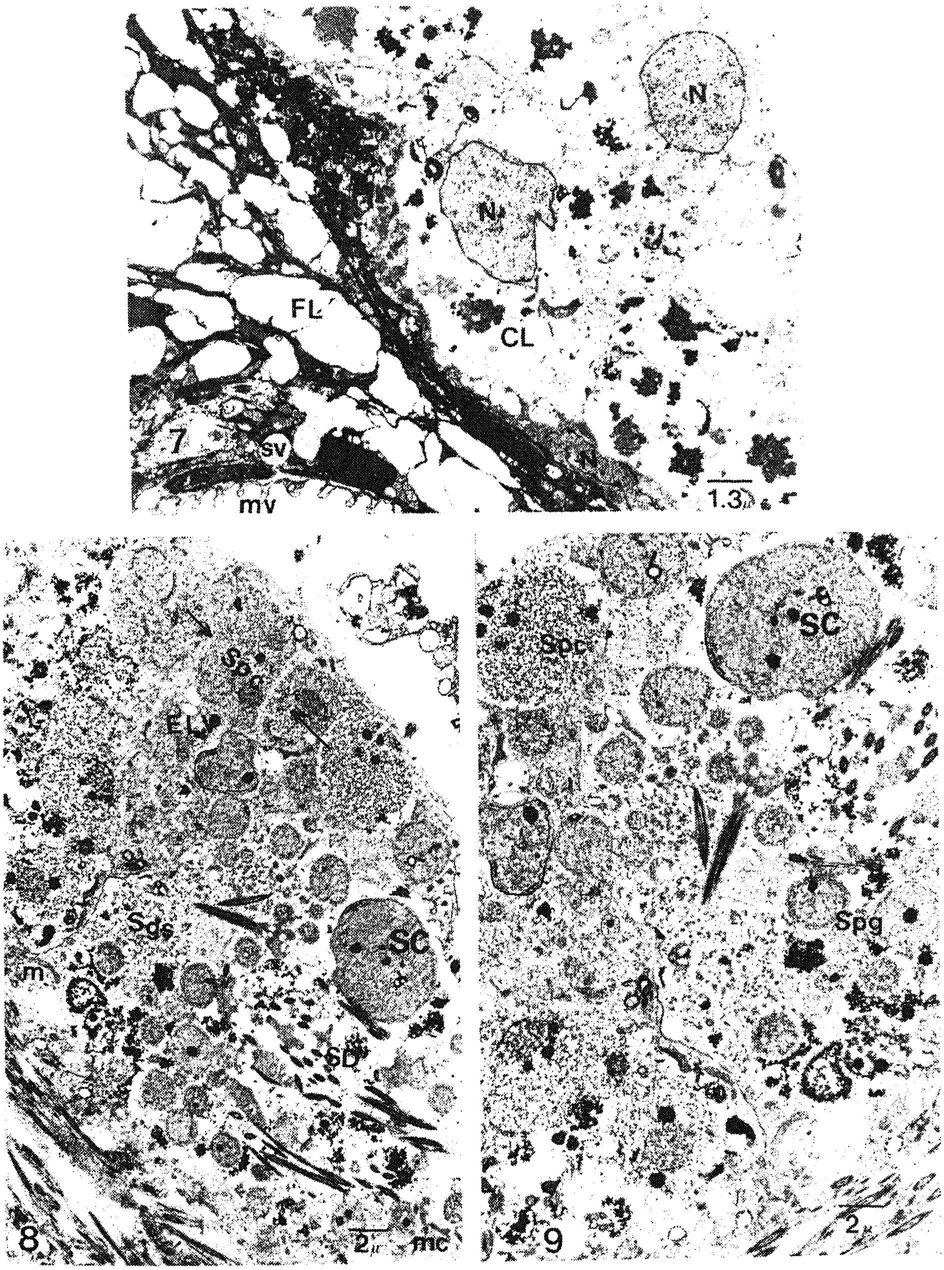
Figures 5 and 6. **5.** Electron micrographs of a whole testes from thick sections showing capsule layer and follicular layer. Apical region shows the spermatogonia (Sg), followed by primary and secondary spermatocytes and spermatids (SD) and spermiogenesis (Spms). Towards the testicular calyx, the mature sperm bundles (SB) occur. Arrows indicate apical region (AR) to middle. **6.** Apical region of the follicular epithelium (FE) with its well developed microvilli (MV) pushing the growing spermatocytes and secretions into the lumen are seen; note the heterophagocytic vesicles (HV). Also note the follicular epithelium with nucleus (N), nerve ending (NE) and electron light vesicles (ELS).



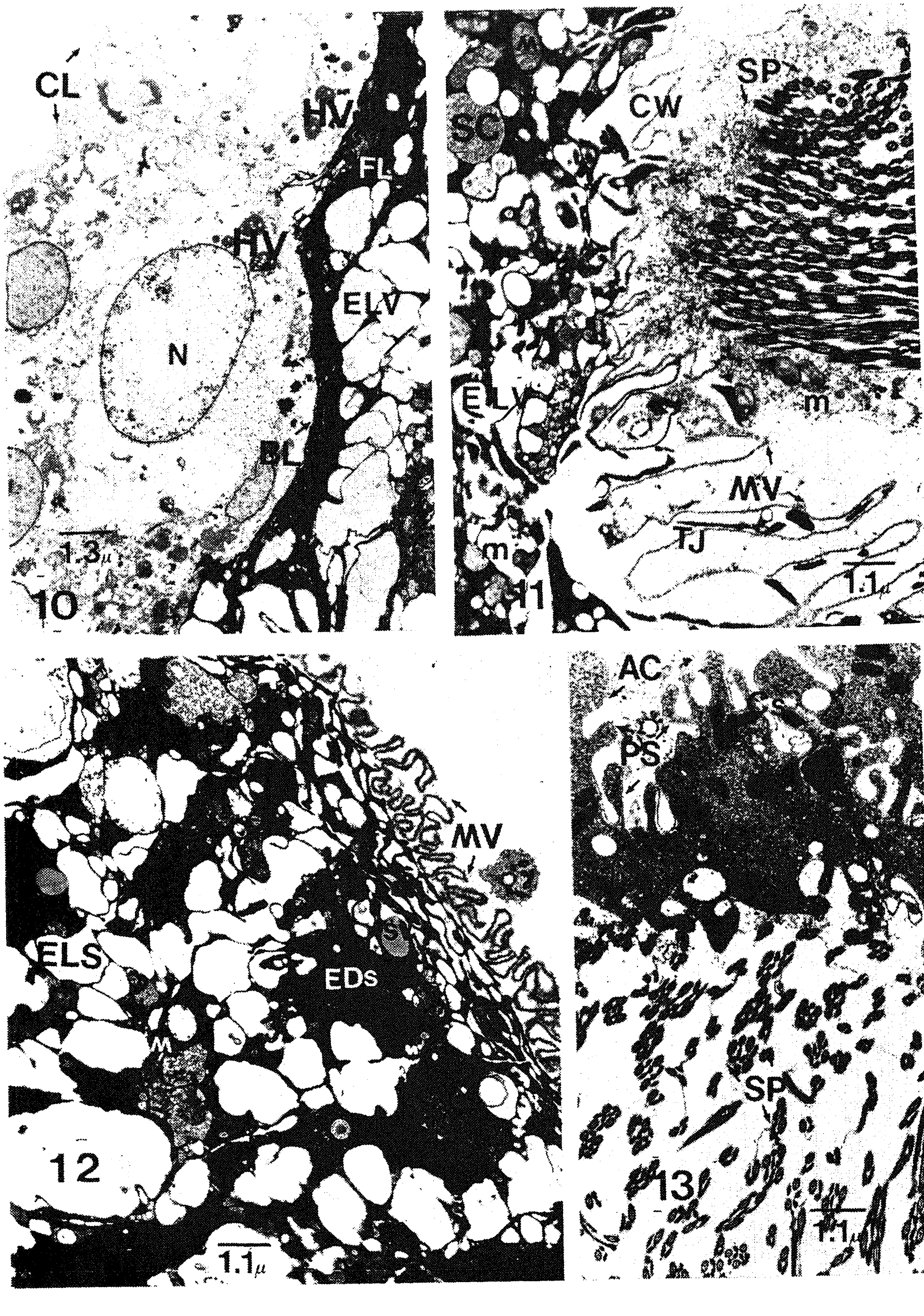
Figures 1–4.



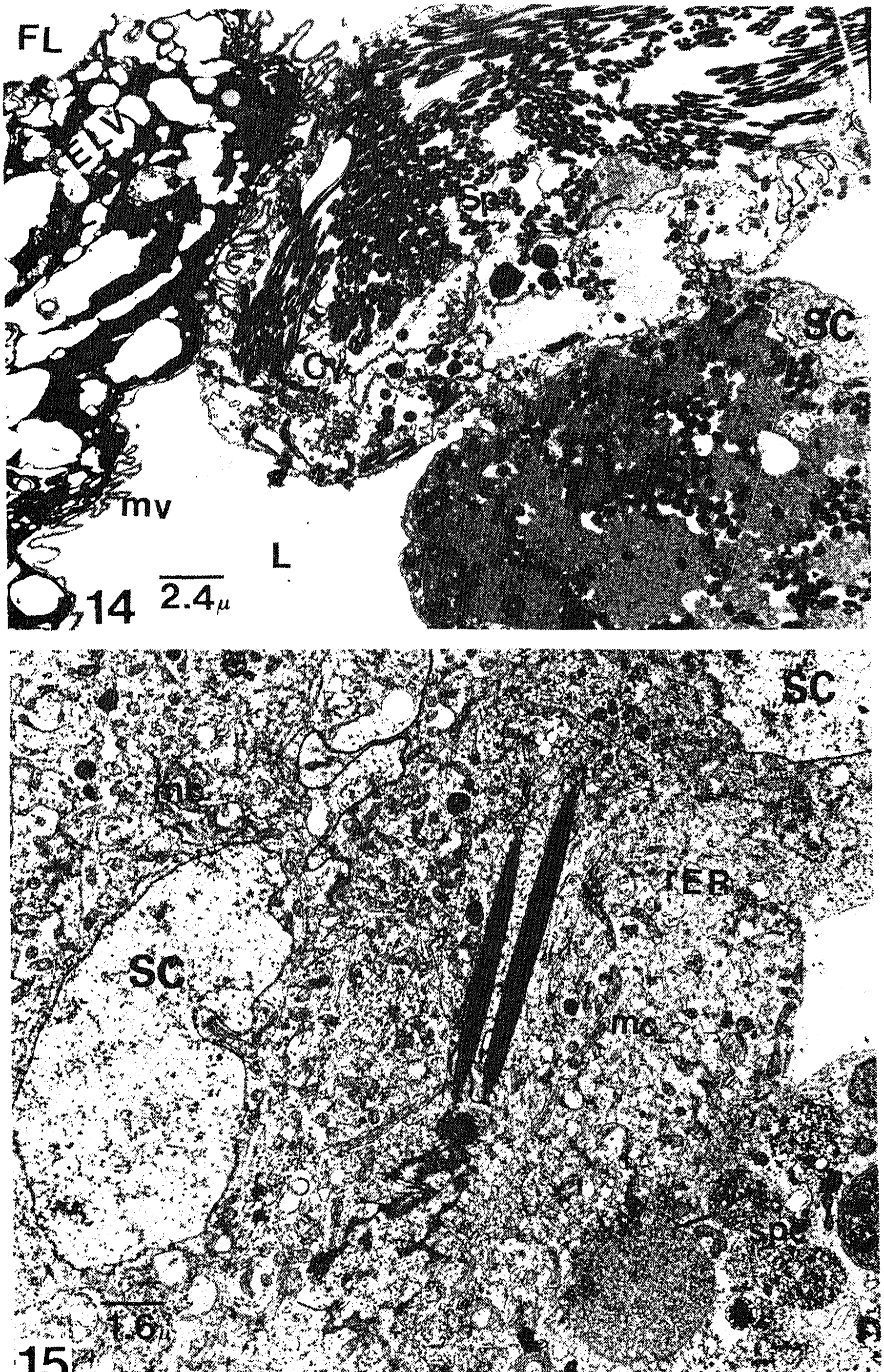
Figures 5–6. For caption, see p. 406.



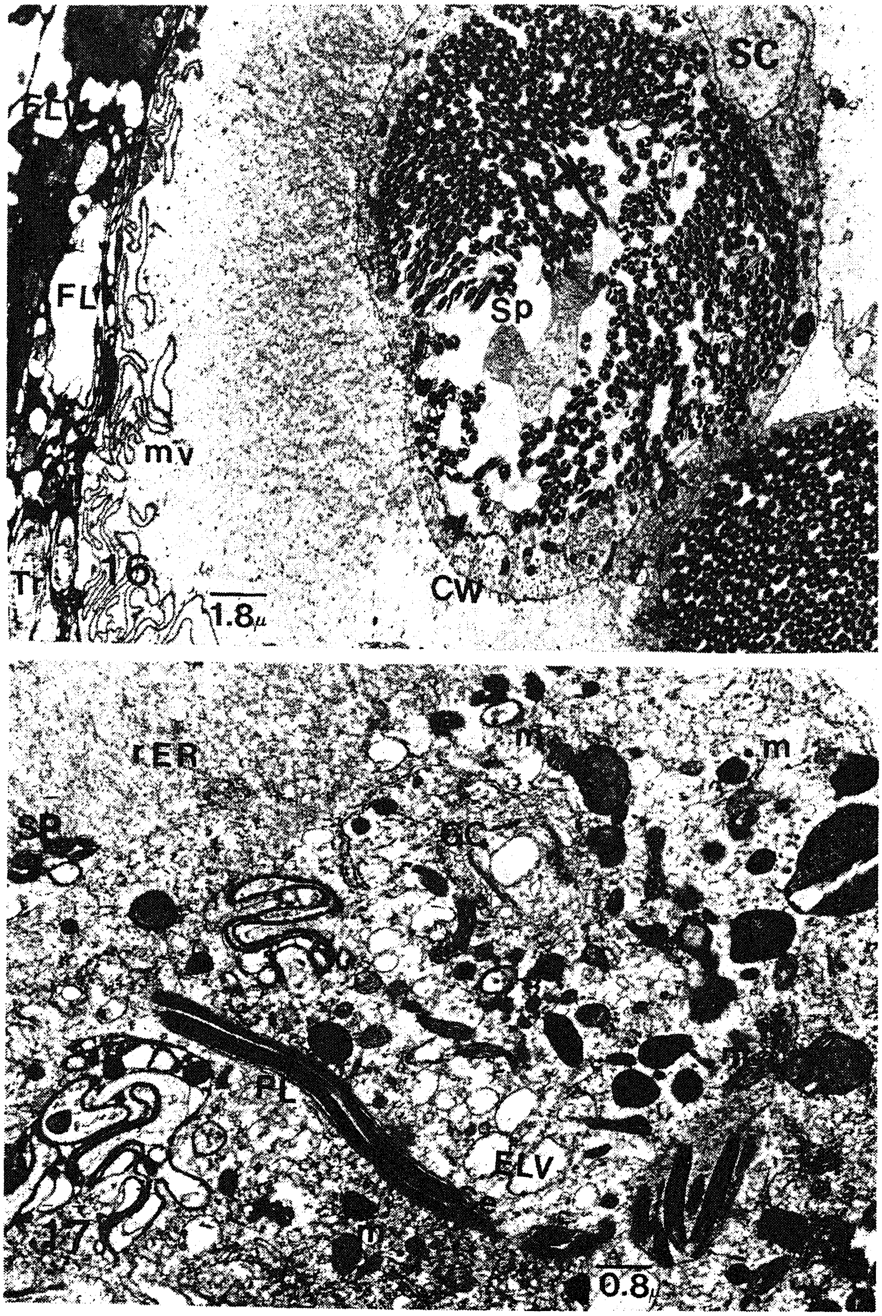
Figures 7–9. **7.** Higher magnification of capsule and follicular layer wherein the capsule layer shows dark dense irregular secretory granules while the follicular layer consists of dense secretory epithelia and secretory vesicles (SV). **8, 9.** Low magnification of apex region of the follicles showing spermatogonial mother cells (SPG), maturing spermatocytes and the sertoli cells (SC), mitochondria (m), mitochondria complex (mc), secretory granules (Sgs) and spermatids (SD).



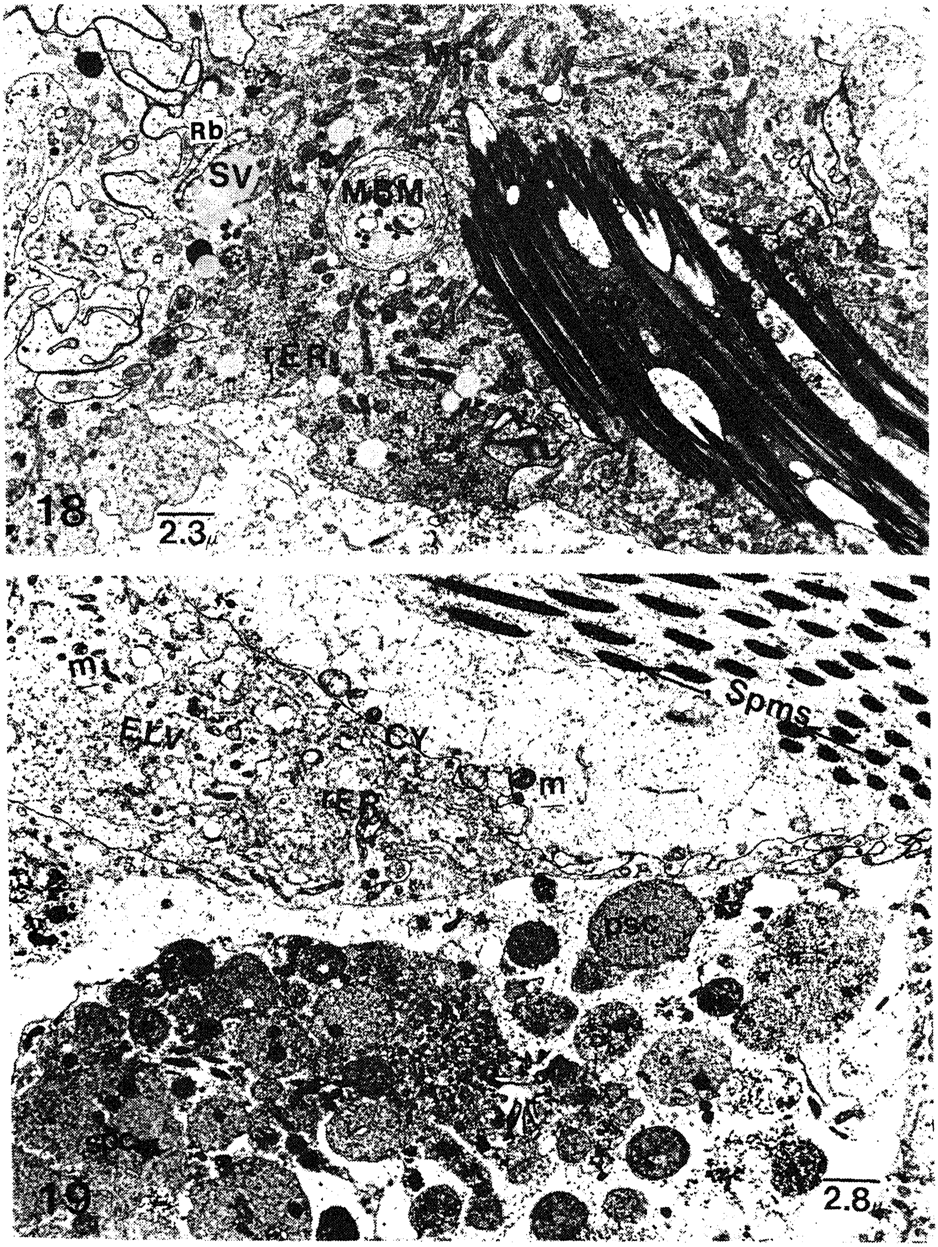
Figures 10–13. For caption, see p. 415.



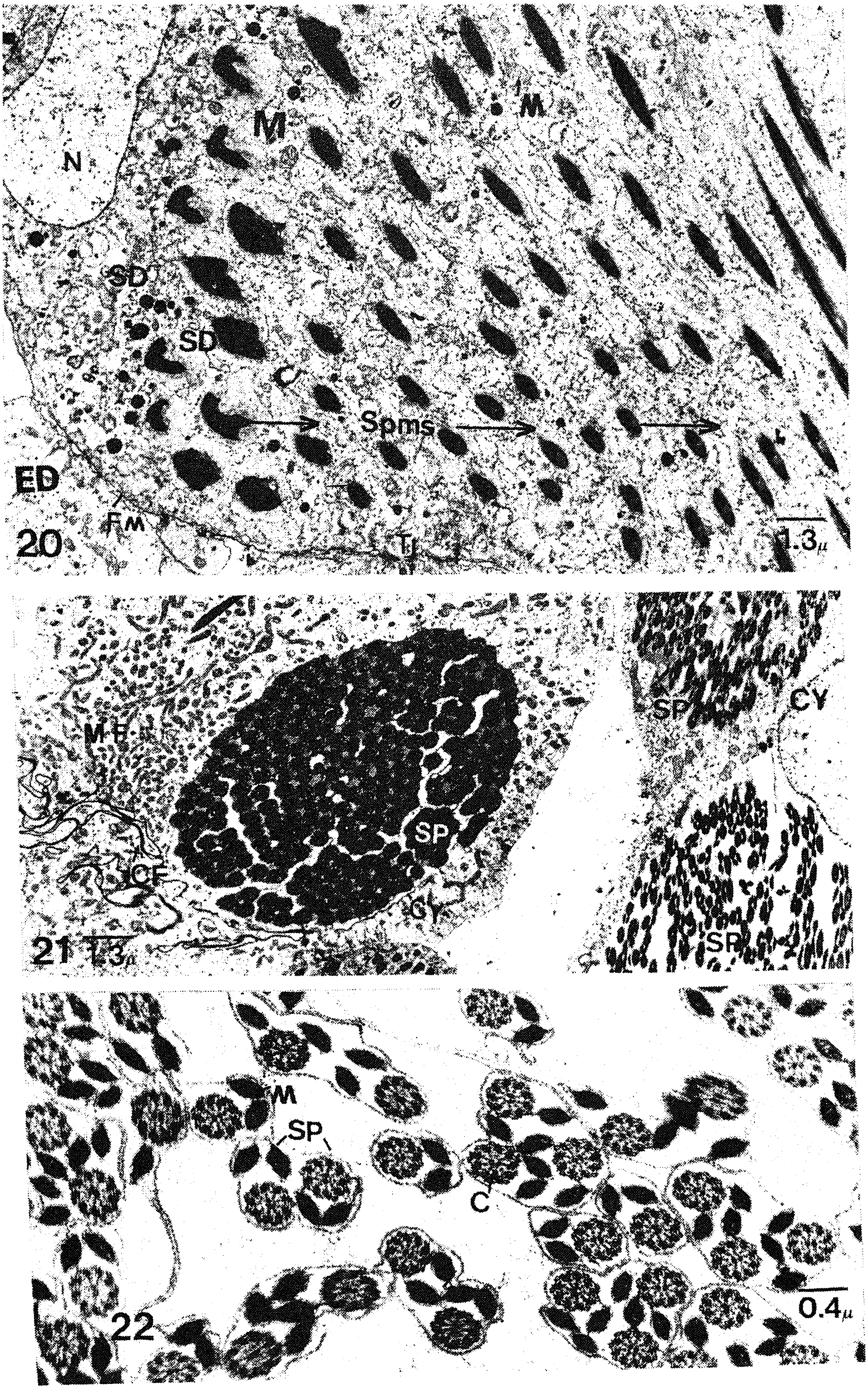
Figures 14 and 15. **14.** Electron micrographs resemble figure 11 in exhibiting the exchange of materials from the follicular epithelium into the cyst and from one cyst to another. **15.** The large sertoli cells are seen in the follicular epithelium and the mc and rER in aiding the formation and the maturity of spermatozoa. Also seen are spermatocytes (spc).



Figures 16 and 17. **16.** The close relation between the cysts is more clearly seen. One of the sertoli cells (SC) is found within cyst. **17.** Maturing spermatozoa and its environment; dark, dense secretory granules, membrane bound dense vesicles, aberrant sperm, ELV and mitochondria. Formation of the flagellum in the developing insect sperm cell may either occur in the late spermatocyte or after the second meiotic division i.e. the early spermatid. In *S. litura* occurs at the former stage, identified by the presence of small mitochondria, Golgi complex (GC) scattered throughout the cell. Prior to their aggregation in the 'Nebenkern'.



Figures 18 and 19. **18.** The germ cell cytoplasm at this stage reflects an undifferentiated condition of the spermatocyte; only a few small cisternae of the endoplasmic reticulum are present (ER), while free ribosomes (Rb) are abundant. The flagellum (Fl) (figure 17) arises in association with one of a pair of centrioles (Ce1, Ce2) placed perpendicularly to each other. Later reorganization of the cell places the flagellar centriole or basal body, behind the nucleus in the elongated cell. Also note membrane bound materials (MBM). **19.** Spermiogenesis (Spms) is seen in the upper cyst and the lower cyst exhibits maturing spermatocytes and in between the follicular epithelium containing rER and n. Also note the spermiogenesis (Spms), primary spermatocyte (psc) and secondary spermatocyte (spc).



Figures 20–22.

has varying length. The cytoplasm is granular and the nuclei are rounded. The lumen is large and has discrete globules. The globules are budded off from the free cell surface into the lumen.

3.1 *Ultrastructure*

The testes are covered by outer capsule layer and an inner follicular layer. The outer capsular layer is distinct and structureless, while the inner layer is very distinct, structured and contains dark and dense cytoplasm. The capsule layer is characterized by the presence of granular cytoplasm, irregular dark and dense secretory vesicles and the latter are seemingly transported into the follicular epithelium. It contains an oval shaped nucleus. Several profiles of heterophagic vacuoles can be seen (figure 10). Lipid droplets are spread over in the capsule layer. Dividing the capsule layer are the basement membrane and basal lamina. The follicular layer consists of an epithelium with dense cytoplasm. The epithelium appears secretory in function in possessing numerous secretory vesicles and granules. It also possesses scattered lipid droplets. There are electron dense and light secretory granules. At the free cell surface of the epithelium towards the lumen, the epithelia exhibit numerous regular microvilli. The secretory materials seemingly collect at the free cell surface and are expelled into the lumen by means of microvilli (figures 11, 12). The epithelium has dark and dense smooth endoplasmic reticulum. Numerous mitochondria occur at the apical region of follicular epithelium. The matrix like materials are collected at the free cell surface in packets (vesicles), which line up and then open into the lumen by extending their tube like structures. This appears like exocytosis mode of secretion taking place (figure 11). A characteristic feature of the testicular follicles is the presence of large cells or nucleated mass of protoplasm in the apex of the germarium (figure 13). This cell is known as an apical cell or versonian cell. This is the region where there are successive stages of development of the germ cells occurring. The upper part of this contains primary spermatogonia and is known as germarium. This is followed by a

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Figures 10–13. **10.** Capsule layer containing the nucleus (N) secretory material, heterophagocytic vesicles overlying the basement membrane and basal lamina (BL). **11.** The apical region exhibits accumulation of secretory materials and transporting them by means of the exocytosis and through microvilli. Cyst wall (CW) also extends itself in the form of pseudopodia in order to exchange secretory material with tight junctions (TJ). **12.** High magnification of follicular epithelium with its texture and secretion and its well defined microvilli. The epithelium exhibits electron dense (EDs) and light (ELS) vesicles, numerous mitochondria, scattered lipid droplets. Notice the accumulation of secretory materials at the free cell surface. **13.** Follicular epithelium showing the spermatogonial mother cell or apical cells (AC) are interconnected by means of protoplasmic strands (PS) or cytoplasmic strands (Cs).

Figures 20–22. **20.** The cyst exhibits the process of spermatogenesis. Its contents and process of elongation of spermatid into spermatozoa. Note the follicular membrane (FM) and tight junction (TJ). **21.** The early spermatid stage and the materials within the cyst are seen. Two other cysts contain maturing spermatozoa. Also note the membrane foldings (MF), cell folding (CF) and cyst (Cy). **22.** Mature spermatozoa within the cyst wall exhibit two large mitochondrial bodies and 9+2 configuration of flagellum. This cyst around mature spermatid may serve as sperm sheath. Also note the centriole (C).

region called zone of growth. The region or zone of growth is where spermatogonia multiplies and usually gets encysted (figures 5, 8, 9, 14–19). The maturation zone follows next where maturation takes place. Finally it is the zone of transformation and here the spermatocytes develop into spermatids completing spermatogenesis (figures 1–3). The spermatids extend and develop a flagellum and thus mature spermatozoa come into existence completing the spermatogenesis process (figures 20–22). The growing spermatogonium, spermatocytes are surrounded by sertoli cells and exchanges are going on at certain points (figures 8, 9). The continued assistance of sertoli cells is very much evident during spermiogenesis (figure 14). The cysts are still surrounded by sertoli cells and there is a communication point of exchange taking place between them. The follicular epithelium is rich in both smooth and rough endoplasmic reticulum, mitochondria complex (figure 15), electron dense and light secretory granules actively take part in the growth and maturing process of spermatogonium, spermatocyte and spermatids. The sertoli cells are prominent by being in close communication with the maturing spermatogonium and spermatocytes. The follicular epithelium exhibits growing and maturing spermatids of different stages marching in and out of the cyst as seen in figure 6. This is a bit puzzling to interpret. For the cyst contains spermatids developing flagellum and the same can be seen between microvilli and the cyst wall. Spermiogenesis completes when the cyst containing mature spermatids develop the flagellum (figure 20). The cyst contains abundant materials that are possibly used by the maturing spermatids. Cell organelles like mitochondria are also present in the act of assisting spermatid developing flagellum. Cysts are interlinked by cytoplasmic strands (figure 21).

4. Discussion

Bright yellow coloured adult testes of *S. litura* are paired and so closely apposed that they appear as a single round organ. The testes of *S. litura* resemble that of other Lepidoptera having enclosed in a common membrane called scrotum. The testes lie dorsally and appear to be held in place by tracheae and strands of basement membrane like material as suggested by Musgrave (1937) and Lai-Fook (1982). This is contrary to earlier reports by Chase and Gilliland (1972) in *Heliothis virescens* and Salama (1976) in *Porthetria dispar* as epithelial strands. Brits (1979a) described as being attached to the epidermis by ligamentum suspensorium and did not explain more. Although reports of several layers surrounding the testes of Lepidoptera have been made, it was found only a single capsule and follicular layers in *S. litura.* Chase and Gilliland (1972) described the tunica externa and interna are nothing other than basement membranes over the capsule and follicular layers. The interfollicular layer is divided into 8 incomplete compartments as described by Brits (1979a) in *Phthorimaea operculella.* The interfollicular layer that divides follicles into compartments are like as in *Calpodes ethlius* (Lai–Fook 1982) and in *Ephestia* (Musgrave 1937). It also bears the pigment responsible for the bright yellow coloured testes. It is clear that spermatogenesis persists in the adult testes, contrary to earlier assertions of Phillips (1970) and Chaudhury and Raun (1966). They reported only spermatids occurring in the adults. Retnakaran (1971) showed through DNA synthesis the possibility division in the adult. Lai–Fook (1982) has actually reported the occurrence of spermatogenesis in the adult *Calpodes ethlius.* The accompaniment of sertoli cells during the various stages

dividing of spermatogenesis and spermiogenesis is significant. The sertoli cells are very similar to Echinoderm interstitial cells in that they provide scaffolding for spermatogenesis. Possibly they phagocytize the waste sperm (Walker 1980; Chia and Bickel 1983; Buchland–Nicks *et al* 1984; Chia and Buchland–Nicks 1987). There are communication points between sertoli cells and maturing spermatocytes; spermatids appear like desmosomes as in Echinoderm testes (Chia and Buchland–Nicks 1987). As regards the blood testes barriers between the cysts and the follicular epithelium, they are like other insects, gastropod, molluscs and mammals in preventing the indiscriminate passage of substances to the developing sperm (Fawcett 1975; Russel and Peterson 1985; Buchland–Nicks and Chia 1986).

Finally, the electron micrographs bring out one key point clearly, i.e. the close relationship between the follicular epithelium and the cyst, and between the cysts themselves. There appears a kind of communal harmony among the stages of development of spermatozoa, i.e. spermatogenesis and spermiogenesis.

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Effect of indomethacin on testicular hypertrophy in hemiorchidectomized immature rats

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Abstract. The effect of indomethacin, an inhibitor of prostaglandin synthesis, on testicular hypertrophy has been studied in immature rats following hemiorchidectomy. Hemiorchidectomy was performed on postnatal day 21 and the weight of the excised testis was recorded. On postnatal day 27, the animals were divided into two groups. Group 1 received saline (0·1 ml) and group 2 received indomethacin orally at 2 mg/kg/day. All animals were treated daily for 15 days. On completion of the treatments, the animals were sacrificed by ether administration. The final body weight and the weight of the remaining testis were recorded. Blood samples were collected by cardiac puncture for testosterone assay. The testes were kept in formol saline. Subsequently, they were prepared for quantitative histological study. Indomethacin significantly decreased the mean diameters of the seminiferous tubules. Cell counts indicated that there was a significant decrease in the mean numbers of spermatogonia, primary spermatocytes and spermatids. The testicular weight gain and the mean testosterone concentrations showed a nonsignificant decrease in treated animals. It is concluded that indomethacin exerts inhibitory action on spermatogenesis in hemiorchidectomized immature rats.

Keywords. Indomethacin; testicular hypertrophy; testosterone; hemiorchidectomy.

1. Introduction

Hemicastration provides a useful model for studying certain physiological aspects of the hypothalamic-pituitary-testicular axis. In immature animals, unilateral castration results in hypertrophy of the remaining testis, increased follicle stimulating hormone (FSH) concentration (Ojeda and Ramirez 1972; Ramirez and Sawyer 1974; Moger 1977; Cunningham *et al* 1978; Selin and Moger 1979) and decreased testosterone concentration (Moger 1977). Luteinizing hormone (LH) concentrations were unchanged after hemiorchidectomy (Ojeda and Ramirez 1972; Selin and Moger 1979).

Since prostaglandins (PGs) were reported to alter the functional integrity of testis (Dev and Mangat 1980, 1982), to reduce testicular weight (Abbatiello *et al* 1975), to inhibit spermatogenesis (Ericsson 1973; Abbatiello *et al* 1976; Tierney *et al* 1979), and may have an important role in regulating testosterone secretion (Memon 1973; Saksena *et al* 1975; Bartke *et al* 1976; Kiser *et al* 1976; Haynes *et al* 1978; Reichard *et al* 1978; Kimball *et al* 1979; Fuchs and Chantharaksri 1981), it was of interest to study the effect of indomethacin, an inhibitor of PG synthesis, on testicular hypertrophy in hemiorchidectomized immature rats.

2. Materials and methods

Immature male albino rats, 21 days old, were used in all experiments. They were selected randomly and kept under controlled conditions of temperature (25 ± 2°C)

and lighting (14 h light/10 h dark). Commercial pelleted food and water were freely available. Hemiorchidectomy was performed under light ether anaesthesia. One testis was removed from each animal, alternating right and left sides for the rats within each group. The initial body weight and the weight of the excised testis were recorded.

The hemiorchidectomized animals (27 days old) were divided into two groups. Group 1 received saline (8·5 g/l, 0·1 ml) and group 2 received indomethacin (Charles E Frosst and Co., Lebanon) orally by gavage at a dose of 2 mg/kg/day. Animals were treated for a period of 15 days.

On completion of experiments, animals were sacrificed by ether administration. The final body weight was recorded. Blood samples were collected by cardiac puncture. Serum was separated and stored at $-20°C$ until assayed for testosterone. The remaining testes were quickly removed, trimmed and their weights were taken before fixing them in formal saline. Fixed testes were sectioned at 6 μm, mounted and stained with haematoxylin and eosin. Seventy seminiferous tubules at the same stage were analyzed for each group. The mean diameters of the seminiferous tubules were measured using ocular micrometer. The mean numbers of spermatogonia, primary spermatocytes and spermatids per tubular cross section were counted. Serum testosterone concentration was measured using Biodata Testosterone Maia Kits (Serono Diagnostics, Milano). The data were analyzed using Student's t test (Steel and Torrie 1960).

3. Results

The initial and final body weights and testicular weight gain are shown in table 1. The final body weight of the indomethacin-treated animals did not differ significantly ($P > 0{\cdot}05$) from the control. The remaining testes of the animals treated with indomethacin showed a nonsignificant ($P > 0{\cdot}05$) decrease in the weight gain when compared to the control.

Indomethacin produced a significant ($P < 0{\cdot}05$) decrease in the mean diameters of the seminiferous tubules, the mean numbers of spermatogonia, primary spermatocytes and spermatids per tubular cross section when compared with the control (table 2).

The mean testosterone concentration in the serum of animals is shown in figure 1. It did not change significantly ($P > 0{\cdot}05$) in indomethacin-treated animals.

4. Discussion

The results of this study demonstrate that indomethacin inhibits spermatogenesis in

Table 1. Effect of indomethacin on body weight and testicular weight gain in hemiorchidectomized immature rats.

Treatment	No. of rats	Body weight (g) Initial	Body weight (g) Final	Testicular weight gain (mg)
Control (saline)	10	31·21 ± 1·44	114·45 ± 4·68	641·39 ± 25·06
Indomethacin (2 mg/kg)	11	35·07 ± 1·85	117·16 ± 3·75	585·35 ± 22·38

Values are mean ± SEM.

Table 2. Effect of indomethacin on the mean diameters of the seminiferous tubules, mean numbers of spermatogonia, primary spermatocytes and spermatids per tubular cross section in hemiorchidectomized immature rats.

Treatment	No. of rats	Mean tubular diameter (μm)	Spermatogonia	Primary spermatocytes	Spermatids
Control (saline)	10	211·78 ± 2·64	95·93 ± 2·52	73·01 ± 4·16	133·05 ± 10·08
Indomethacin (2 mg/kg)	11	143·12 ± 0·98*	83·21 ± 2·11*	51·86 ± 3·79*	60·93 ± 8·09*

Values are mean ± SEM.
*Significantly different ($P < 0{\cdot}05$) from control.

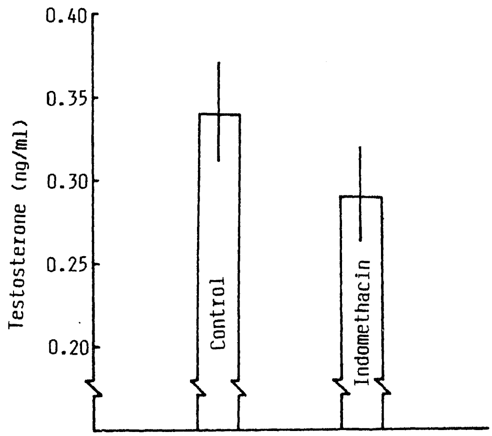

Figure 1. Effect of indomethacin on serum testosterone concentration in hemiorchidectomized immature rats. The height of each bar indicates the average amount (± SEM) of testosterone.

hemiorchidectomized immature rats. It caused a significant decrease in the mean diameters of the seminiferous tubules, the mean numbers of spermatogonia, primary spermatocytes and spermatids. These results seem to agree with the results reported by Scott and Persaud (1978). These authors found that the inhibitor of PG synthesis, acetylsalicylic acid, produced a significant decrease in the mean diameters of the seminiferous tubules, the mean numbers of preleptotene and pachytene spermatocytes and spermatids when administered at a dose of 150 mg/kg for a period of 12 days. On the other hand, indomethacin at a dose of 1 mg/kg twice a day for 15 days produced a pronounced increase in spermatogenesis in the mouse (Abbatiello *et al* 1975). The discrepancy between our results and those reported by Abbatiello *et al* (1975) could be due to differences in doses being used and differences in the age of animals. The inhibitory effect of indomethacin on spermatogenesis may be indirect through inhibition of PGs synthesis in the pituitary gland. The possibility of pituitary effect of indomethacin is based on the suggestion of Sato *et al* (1975) that indomethacin acts on the pituitary to reduce its PG biosynthesis and/or release, and the evidence that variety of PGs have been reported to stimulate both FSH and LH secretion *in vivo* (Hedge 1977). Since FSH is responsible for maintenance of spermatogenesis (Ganong 1985) and hemiorchi-

dectomy in immature animals results in an increased FSH concentration (Ojeda and Ramirez 1972; Ramirez and Sawyer 1974; Moger 1977; Cunningham *et al* 1978; Selin and Moger 1979), it was suggested, on the basis of these findings and the present results, that the inhibition of spermatogenesis in hemiorchidectomized immature rats treated with indomethacin was brought about by decrease in FSH concentration.

Serum testosterone concentration did not change significantly in animals treated with indomethacin, although a decrease was observed. The inability of indomethacin to decrease significantly serum testosterone concentration may be related to the LH production. Since LH is known to stimulate testosterone secretion by the interstitial cells of Leydig (Ganong 1985) and its concentration did not change following hemicastration (Ojeda and Ramirez 1972; Selin and Moger 1979), it is possible to postulate that the decreased cellular sensitivity to luteinizing hormone releasing hormone caused by inhibition of PG synthesis (Sato *et al* 1975) was not sufficient to reduce LH concentration significantly, although a clear picture concerning changes in LH is lacking in the present investigation. It is concluded that indomethacin exerts inhibitory action on spermatogenesis in hemiorchidectomized immature rats.

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Rates of swarming and absconding in the giant honey bee, *Apis dorsata* F.

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Abstract. The frequency and timing of swarming and absconding of the giant honey bee *Apis dorsata* F. at 5 perennial nesting sites located in and around Bangalore district during 1986–88 are determined. The percentages of swarming and absconding are very high and both these migratory activities occur regularly and at definite times of the year. Swarming was noticed both during October–November and in April, while absconding was observed during May to July. Swarming and absconding together caused migration in *Apis dorsata*.

Keywords. *Apis dorsata*; swarming; absconding; honeybee; rockbee.

1. Introduction

The giant honeybee *Apis dorsata* F. still exists as a wild bee (Beeson 1941; Lindauer 1957; Abrol 1985). This species builds its single large comb in an open place suspended from branches of tall trees, big rocks, water tanks and buildings (Deodikar *et al* 1977; Chandrasekhara Reddy 1983; Ahmed and Abbas 1985). Although its nests are sometimes found singly, the rockbee is highly gregarious and nests in groups ranging from a few to few hundreds nests (Shankar Reddy 1988). It is highly migratory in nature and normally moves from place to place. Only some of the reasons for such migration are known (Sihag 1982).

General information on migration, swarming and absconding are available for African and European bees (Attridge 1917; Demuth 1921; Allen 1956; Smith 1960; Martin 1963; Simpson and Riedel 1963; Butler 1967; Butler and Simpson 1967; Simpson 1973; Burgett 1974; Fletcher 1975, 1976, 1978; Fletcher and Tribe 1977; Fell *et al* 1977; Winston *et al* 1979; Caron 1979, 1981; Lensky and Seifert 1980; Page 1981). However, no detailed information on the nature of swarming, absconding and migration in the giant honeybee *A. dorsata*, the largest honey producing species in India is available. This paper reports the results of a two year study on the frequency and timing of absconding and swarming and their possible relationship with migration.

2. Materials and methods

Five perennial multiple colony nesting sites were selected for studying swarming and absconding behaviour. These nesting sites are located in the Bangalore district.

2.1 *Swarming*

All 5 nesting sites were observed weekly for the presence of occupied and deserted

combs and daily for the emergence and settlement of swarms. The total numbers of swarms issued, swarms settling within the nesting sites, swarms leaving their respective nesting sites and swarms coming from other nesting sites were recorded. The size of the population of each colony and the variation in colony number at each nesting site were recorded to classify the swarms as immigrant and emigrant swarms.

2.2 *Absconding*

Departure of any colony without emergence of the new queen(s) or over-crowding of bees was considered as absconding. All colonies were observed weekly and the total number of colonies absconded in all 5 nesting sites during the study period was recorded.

3. Results

Number of colonies, both live and deserted in each of the 5 nesting sites for each month are shown for two years in table 1. Live colonies were present in all 5 nesting sites throughout the period 1986–88 except at nesting site-1 in July. However, deserted colonies were noticed only during May to July. The number of live colonies varied from 0–108, while the number of absconding colonies varied from 0–54.

Data on the numbers of occupied and deserted combs at the 5 nesting sites were subjected to ANOVA (table 2). Our results show that the variation between the groups is significant ($P < 0.01$). Further, on subjecting these data to Tukey measure we find that the mean differences between groups 2 and 1, 2 and 3, 2 and 4 for occupied combs and groups 2 and 1, 2 and 3, 2 and 4, 2 and 5 for deserted combs are significant. However, the mean differences between all other groups are not significant (table 3).

Table 4 shows the incidence of swarming and absconding for the period 1986–88. A total of 336 colonies were observed and the percentage of absconding and swarming ranged from 81–100 and 53–108 respectively of the total colony population of all nesting sites. The number of swarms settled within the respective nesting sites were greater than emigrant swarms. Emigrant swarms were noticed in nest site 3–5 and the percentage varied from 8–28 of the total swarms issued, while immigrant swarms were noticed in nesting sites 1 and 2 with a minimum and maximum per cent of 19 and 29 respectively. The minimum and maximum per cent of swarms settled within the respective nesting site were 53 and 81 respectively.

The percentage of colonies absconding or swarming in each month for the two years are shown in figure 1. The period of absconding was almost same in both the years except that maximum per cent in the first year was 79 when compared to 53 in the second year. The occurrence of swarming during 1986–87 and 1987–88 showed variation in peak per cent activity; there were two peaks of swarming during 1986–87 against three during 1987–88. Peak swarming activity was observed during November and April for the year 1986–87; October, January and April for the year 1987–88.

Table 1. Monthly occupied and deserted colonies of *A. dorsata* during 1986–88.

	Nest site number									
	1		2		3		4		5	
Month	1986–87	1987–88	1986–87	1987–88	1986–87	1987–88	1986–87	1987–88	1986–87	1987–88
August										
Occupied	2	3	10	8	5	6	3	5	8	7
Deserted	0	0	0	0	0	0	0	0	0	0
September										
Occupied	3	5	10	8	5	8	3	8	9	7
Deserted	0	0	0	0	0	0	0	0	0	0
October										
Occupied	3	7	8	7	8	13	4	8	11	13
Deserted	0	0	2	1	0	0	0	0	0	0
November										
Occupied	5	8	8	7	11	13	6	11	14	16
Deserted	0	0	0	0	0	0	0	0	0	0
December										
Occupied	5	10	15	9	13	13	7	12	14	18
Deserted	0	0	0	0	0	0	0	0	0	0
January										
Occupied	5	13	35	9	13	15	7	15	13	20
Deserted	0	0	0	0	0	0	0	0	1	0
February										
Occupied	6	13	35	25	13	16	7	16	13	20
Deserted	0	0	0	0	0	0	0	0	0	0
March										
Occupied	6	14	38	42	18	19	9	18	21	25
Deserted	0	0	0	0	0	0	0	0	0	0
April										
Occupied	7	15	72	108	25	22	13	20	26	28
Deserted	0	0	0	0	0	0	0	0	0	0
May										
Occupied	4	8	58	51	18	14	9	14	14	19
Deserted	3	7	14	54	7	8	4	6	12	9
June										
Occupied	1	4	35	20	9	8	5	7	9	8
Deserted	2	3	22	31	9	6	4	7	5	11
July										
Occupied	0	2	8	8	3	5	1	3	5	5
Deserted	1	2	27	12	6	3	4	4	4	3

4. Discussion

Our results indicate that the variation in the number of occupied and deserted combs between the 5 nesting sites during 1986–88 is significant, the nest density is high and that approximately the same number of live colonies are always present inspite of heavy migration.

Table 2. ANOVA table of a 5 group design of occupied and deserted colonies of *A. dorsata*.

	Source of variance	
	Between groups	Within groups
Sum of squares		
Occupied	5489·36	18597·44
Deserted	2267·8	1280·5
Degrees of freedom		
Occupied	4	115
Deserted	4	25
Mean square/variance		
Occupied	1372·34	161·716
Deserted	566·95	51·22
F		
Occupied	8·486	—
Deserted	11·069	—

Table 3. Tukey's measure for data in table 1.

		X_1	X_2	X_3	X_4	X_5
X_1						
Occupied	6·208	—	19·583*	6·0	2·583	8·084
Deserted	3	—	23·833*	3·5	1·83	4·33
X_2						
Occupied	25·791	19·583*	—	13·583*	17*	11·499
Deserted	26·833	23·833	—	20·333*	22·003*	19·503*
X_3						
Occupied	12·208	6·0	13·583*	—	3·417	2·084
Deserted	6·5	3·5	20·333*	—	2·33	0·83
X_4						
Occupied	8·791	2·583	17*	3·417	—	5·501
Deserted	4·83	1·83	22·003*	2·33	—	2·5
X_5						
Occupied	14·292	8·084	11·499	2·084	5·501	—
Deserted	7·33	4·33	19·503*	0·83	2·5	—

Occupied HSD = 12·246; deserted HSD = 15·105.
*Mean difference are significant.

The timing of swarming in all 5 nesting sites confirms that swarming takes place at a particular time of the year. Swarming in honey bees is a natural way of population dispersal and colony multiplication.

Very high incidences of absconding in all 5 nest sites indicate that absconding is an annual phenomenon and is observed during May to July each year. Similar behaviour has been observed in other species of honeybees (Fletcher 1975, 1976; Woyke 1976). Swarming and absconding together cause extensive migration in *A. dorsata*.

Table 4. Annual percentage of swarming and absconding in different nest sites of *A. dorsata* during 1986–88.

Nest site Number	Year	Number of colonies observed	Swarms settled within the nest sites (%)	Immigrant swarms (%)	Emigrant swarms (%)	Total per-cent of swarms issued	Absconding (%)
1	1986–87	7	71	29	0	71	100
	1987–88	15	53	27	0	53	87
2	1986–87	72	69	19	0	69	94
	1987–88	108	65	28	0	65	93
3	1986–87	25	80	0	28	108	68
	1987–88	22	73	0	23	96	77
4	1986–87	23	77	0	15	92	92
	1987–88	20	75	0	15	90	85
5	1986–87	26	81	0	8	89	81
	1987–88	28	75	0	14	89	82

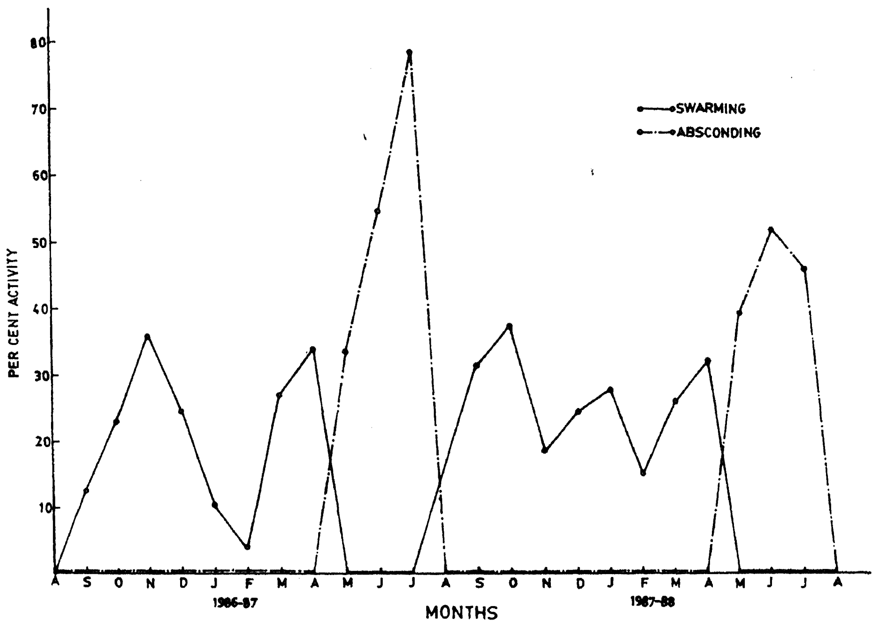

Figure 1. Monthly per cent of swarming and absconding behaviour of *A. dorsata.*

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Factors affecting the surface cast production by some earthworms of Indian tropics

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Abstract. Forty one sites in mango gardens located in different districts of southern Karnataka were selected for the study during September 1984. Rate of surface cast production, earthworm density and species composition of each site were recorded and compared in relation to the soil moisture, pH, temperature, organic matter and soil type of each site. Only 19 out of the 41 sites examined showed surface casting. Rate of surface cast production is dependent on soil, pH, temperature, soil type and organic matter content. The bulk density of the soil established a direct linear correlation, and the field capacity of soil, a perfect U-shaped (parabolic) relationship with the rate of surface cast production. Sandy loams had greater species diversity as well as population density than the clays. Worms were not found in heavy sands and acid soils. Positive correlations were obtained between organic matter and species diversity or density; other factors were poorly correlated. A positive correlation between the cast output and the relaxed length of the worm was obtained. It is discussed that the field capacity and bulk density of soils are the determining factors for variations in surface cast production.

Keywords. Tropical earthworms; surface cast production; field capacity; bulk density of soil; wormcast output.

1. Introduction

In south Indian humid tropics, despite their ubiquitous occurrence in subterranean soils, the earthworms produce surface casts only at certain sites. It was formerly believed that surface casting species of earthworms are quite different from those of non-surface or burrow casters. Of late such a belief is slowly receding as all worms are known to cast on the surface depending on the edaphic and climatological conditions, especially in sub-humid tropics. The production of surface casts depends on the ecological categories (way of life) and community structure of the worms. If anecics (deep soil burrowers) are absent, some endogeics (sub-soil dwellers) are known to produce surface casts. *Eisenia rosea*, which was once regarded as non-surface-caster is now shown to produce surface casts when the bulk density, organic matter and fine silt particles of the soil are favourably increased (Thompson and Davies 1974). Recent reports further emphasize the fact that the bulk density causing the compaction of soils either induces or affects the surface casting by the worms (Thompson and Davies 1974; Piearce 1984; Habibullah and Ismail 1985). In sites which experience periodical drought or water logging or even occasional wetting of top soils, the surface casting is seldom observed. However, the factors affecting surface casting by the earthworms are not clearly elucidated. This paper reports particularly how the moisture level at the field capacity of soil of the site would affect the rate of surface cast production by the inhabiting earthworm populations.

2. Materials and methods

2.1 *Sites and field experiments*

Mango (*Mangifera indica*) gardens (varying in area between 2–6 ha each) located in different districts of Karnataka state, were selected for the study. The garden sites vary greatly in agroclimatological and edaphic factors, and floristic and faunistic composition. Many of the sites suffer occasional interferences by human as well as by stray cattle. In September 1984 (as September is supposed to have a peak rise in worm populations, see Krishnamoorthy 1985) ten, one-square meter field plots were marked at random from each garden. Grasses, weeds and stubble at the plot were cut, and the plots were also cleared of existing earthworm casts. The wormcasts produced in 3 successive days were collected, pooled, dried at 105°C overnight, and weighed. The worm density was noted after excavating one cubic metre soil in each plot and sorting out the worms of all ages and sizes by hand. The worms were identified and for convenience the taxonomy and nomenclature of Stephenson (1923, 1930) was used. The averages of the 10 random plots chosen were noted.

Random soil cores from each garden, were taken from 20 cm depth for pH measurements in aqueous suspension, moisture content as weight loss on drying at 105°C, and organic matter as percentage weight loss of oven dried (at 105°C) samples on ignition at 650°C in a muffle furnace. Standard correction for loss of hygroscopically bound water (from mineral soil fractions) mainly according to clay content was applied. The soils of the plots were classified according to Mirajkar *et al* (1979). Bulk density of the soil samples was assessed according to Brady (1974).

Surface cast in the field was collected as described earlier (Krishnamoorthy 1985) and the rate of surface cast production (RSCP) in an unit area was assessed after noting the average 24 h output.

Field capacity (FC) is the maximum amount of water which a freshly drained soil can hold. Water content of a soil varies between wide limits. For several practical purposes a determination of field capacity is more meaningful than the water content, as the former does not depend on the weather conditions just before sampling. Moisture content at field capacity of soil at the selected sites was determined gravimetrically using a simple corner type sampler by cutting off the roll-edged end of a cylindrical (22 cm tall × 9 cm dia) metal can. The other end was perforated and the soil was sampled (Bennett and Humphries 1974).

Meteorological data were obtained from the nearest station.

2.2 *Laboratory maintenance of worms*

About 500 individuals of each of the common species collected from the selected sites were stocked in the laboratory in wooden boxes (30 × 20 × 15 cm) with a nylon (1 mm mesh) mosquito net underneath serving as a bolting cloth. Each box contained 8 kg of dry, rich and garden-loam amended with well measured leaf compost (in 1 : 1 w/w). The amendment was earlier sieved to pass through a 5 mm mesh. Approximately 2·24 l of water was added to this soil amendment to give a mean moisture content of about 28%, and the boxes were covered with black cloth protecting the latter from light.

The RSCP of species or an ecological community was studied in the following way under laboratory conditions: Garden-loam (amendment) soil whose dry weight was already known, was packed in a PVC cylinder (20 cm height, 11 cm diameter) to a desired height. One worm individual from each of 10 species listed in table 3 were introduced into this cylinder and left undisturbed in a dark place over a period of 10 days. Production of surface cast was observed over this period, and every day the surface castings were collected, dried at 105°C, and weighed. The average of 10 days was taken as worm cast output per day. Considering the weight of the soil packed, and the volume of the column, the bulk density of the soil pack was calculated.

Measurements of cast output by individual species were made using the same soil packed in PVC cylinders in the following way: Each cylinder was 14 cm long and 8 cm diameter with both ends open. After packing it with soil, the bottom end was sealed with a nylon-(mosquito) net cloth, and the other end was left open. A portion of 750 g dry soil was placed at first in each cylinder, compressed to a depth of 11 cm, a further 253·22 g of dry soil added and the whole soil column compressed to 12 cm. A volume equivalent to 180·58 g water was then added, giving a mean moisture content of 18% and bulk density of 1·6625 g/cm^3. Ten adults of each species mentioned in figure 6 were released into the soil of each cylinder, and the surface castings were collected for 3 successive days. They were pooled, dried at 105°C overnight and weighed to assess the rate of species cast output.

2.3 *Statistical analyses*

Regression analyses of the data were according to Snedecor and Cochran (1967).

3. Results

Table 1 summarises the data collected from 41 sites in mango gardens located in different districts of southern Karnataka. These data relate to the soil characters like bulk density, moisture level at field capacity, and total soil moisture, earthworm densities, and the rate of surface cast production. Each site was inhabited by two or more species, and each locality by more than one species. It is clear from table 1, the surface cast production did not occur in the sites where the bulk density of soils was below 1·30 g/cm^3. Out of 41 sites examined only 19 (46·34% of the total) had shown the surface cast production. The total surface cast production varies from site to site. All the 41 sites were inhabited by worms, only in two sites the worm densities were measured as low as 2 and 3 per m^3 of the soil; the highest recorded was 198 per m^3. The soil moisture content of the sites varied on a wide scale; most of the sites had more than 5% soil moisture. In 3 sites the latter varied between 3 and 5 at the time of sampling. Surprisingly one site which was having a soil moisture level of 3·52% had a worm population of 26 per m^3 (table 1).

In order to emphasize the relationship between the individual factor and the rate of surface cast production, regression analyses of the data were done (table 2). There was a significant negative correlation between the soil moisture content and the rate of surface cast production (figure 1, table 2). Significant positive correlations existed between the soil moisture content and the population density. The population

Table 1. The abundance and RSCP of earthworm species of mango (*M. indica*) gardens of different agroclimatic sites of Karnataka, with reference to soil type, bulk density, field capacity and total soil moisture conditions during September 1984.

Location		Soil type	No. of species collected	Species composition in the order of abundance	BD (g/cm^3)	FC (%)	Soil			Density (No/m^3)	Adult body weight (g/10 worms)	RSCP (mg dw day^{-1} worm^{-1})
Area	Site						Moisture (%)	Temp. (°C)	Organic matter (%)			
Kolar	1	BSL	6	Lm, Pc, Pel, Db, Os, Op	1·02	22·60	10·25	22	4·28	56	29·6	0
	2	BSL	6	”	1·03	34·30	17·34	23	3·75	83	71·8	0
	3	BSL	4	”	1·44	24·25	12·46	22	3·62	88	71·4	380
	4	BSL	6	”	1·12	31·40	15·33	24	2·62	98	72·8	0
	5	BSL	6	”	1·48	20·75	9·28	23	4·01	82	61·9	575
Chitradurga	1	BSL	5	Pc, Pel, Os, Ob, Im, Db	1·20	21·21	4·81	24	4·01	16	12·2	0
	2	BLSL	6	”	1·51	38·05	20·22	25	3·86	87	69·3	617
	3	BCL	4	”	1·73	17·24	4·57	24	2·91	42	31·6	1340
	4	BSL	5	”	1·30	21·13	5·23	24	1·68	3	6·2	0
	5	LSL	6	”	1·08	29·16	16·32	24	3·68	82	72·6	0
Kollegal	1	BLSL	4	Lm, Op, Oo, Ob, Db, Pel	1·07	34·36	18·26	25	5·62	98	81·4	0
	2	BCL	6	”	1·58	19·35	7·32	25	6·26	36	22·3	787
	3	LSL	6	”	1·36	33·60	15·55	25	6·36	63	61·2	241
	4	BSL	5	”	1·11	31·36	14·39	24	5·26	72	63·1	0
Mysore	1	BSL	4	Pc, Pel, Lm, Oo, Ob, Db	1·13	21·62	5·11	23	1·82	2	12·1	0
	2	BSL	3	”	1·33	33·60	26·12	22	1·92	118	91·3	223
	3	LSL	4	”	1·14	46·20	23·82	21	2·68	131	82·3	0
	4	LSL	5	”	1·40	41·45	25·71	21	4·28	142	86·4	340
	5	BSL	6	”	1·30	26·05	15·20	22	6·31	51	38·7	282

Kanakapura	1	BSL	4	Pel, Pc, Lm, Oo, Db, Ob, Pex	1·21	26·32	7·34	22	2·38	23	16·3	0
	2	BCL	5	"	1·92	16·20	3·52	21	2·16	26	18·7	1862
	3	BSL	4	"	1·22	29·91	9·94	22	3·08	35	12·3	0
	4	BLSL	6	"	1·46	38·80	22·63	21	3·09	68	49·1	456
	5	LSL	7	"	1·15	48·63	20·19	23	3·62	88	72·3	0
Tumkur	1	BSL	4	Lm, Pm, Db, Pel	1·13	36·12	9·71	21	4·31	27	17·3	0
	2	BSL	3	"	1·19	26·16	8·73	21	4·39	26	17·1	0
	3	BLSL	4	"	1·08	29·98	13·18	22	5·62	53	29·3	0
	4	BCL	4	"	1·61	42·50	19·89	22	6·78	73	56·7	715
Mandya	1	BSL	3	Db, Lm, Pel, Pc	1·32	28·65	20·78	24	4·38	97	51·3	145
	2	BSL	2	"	1·18	32·62	21·91	23	4·62	98	68·8	0
	3	BCL	4	"	1·59	40·61	17·38	22	4·72	107	91·4	861
Hassan	1	BSL	5	Ga, Lm, Pel, Db, Op	1·41	36·05	25·73	20	5·38	127	92·3	305
	2	BSL	4	"	1·13	38·81	26·83	19	5·87	129	84·6	0
Chikmagalur	1	BSL	6	Db, Ga, Lm, Pel, Op, Un	1·32	35·35	27·47	19	6·32	156	84·8	91
	2	BSL	5	"	1·11	46·28	28·80	18	6·86	152	141·1	0
Madikeri	1	BSL	6	Ga, Lm, Pel, Op, Pm, Db	1·02	48·18	21·22	18	6·32	109	92·4	0
	2	BSL	5	"	1·04	40·13	22·76	20	4·26	108	91·8	0
	3	BSL	6	"	1·31	33·58	31·41	21	3·92	198	121·3	59
Bellary	1	BSL	3	Lm, Pel, Pc	1·68	17·45	5·35	25	2·68	33	14·3	1143
	2	BLCL	2	"	1·88	17·20	4·38	24	3·26	41	22·4	1559
	3	BLSL	1	"	1·11	28·16	11·24	25	3·16	52	32·5	0

BSL, Brown sandy loam; SL, sandy loam; BCL, brown clayey loam; BLSL, black sandy loam; LSL, light sandy loam; BLCL, black clayey loam; BD, bulk density; FC, field capacity; Lm, *Lampito mauritti* Kinberg; Pc, *Pontoscolex corethrurus* Fr. Mull; Pel, *Pheretima elongata* E Perrier; Oo, *Ocnerodrilus occidentalis* Eisen; Db, *Drawida barwelli* Beddard; Op, *Octochaetona pattoni* Michaelsen; Pex, *Perionyx excavatus* E Perrier; Ob, *Octochaetoides beatrix* Beddard; Os, *Octochaetoides surensis* Michaelsen; Ga, *Glyphidrilus annandalei* Michaelsen; Pm, *Perionyx mysorensis* Stephenson.

Table 2. Regression equations and statistical comparisons of earthworm distribution and the RSCP with reference to edaphic factors of mango gardens surveyed.

Parameters compared (x vs y)	Relationship examined	Regression equation	Correlation coefficient (r)	Level of significance
Soil moisture vs RSCP	Linear	$y=1414{\cdot}44-47{\cdot}28x$	$-0{\cdot}8138$	$P<0{\cdot}01$
Worm density vs RSCP	Linear	$y=1301{\cdot}15-7{\cdot}79x$	$-0{\cdot}7016$	$P<0{\cdot}01$
Field capacity vs RSCP	Parabola	$y=7760-506x+8{\cdot}2x^2$	0·7558*	$P<0{\cdot}01$
Bulk density vs RSCP	Linear	$y=2666x-3384$	0·9416	$P<0{\cdot}01$
Bulk density vs worm density	Linear	$y=271{\cdot}1+3{\cdot}53x$	0·3118	NS
Field capacity vs worm density	Linear	$y=14{\cdot}99+0{\cdot}13x$	0·4812	NS
Moisture vs worm density	Linear	$y=5{\cdot}20x-4{\cdot}80$	0·9181	$P<0{\cdot}01$
Biomass vs RSCP	Linear	$y=1317-11{\cdot}62x$	$-0{\cdot}6988$	$P<0{\cdot}01$
Worm density vs body weight	Linear	$y=7{\cdot}01+0{\cdot}62x$	0·9412	$P<0{\cdot}01$

NS, Not significant. *r^2 value.

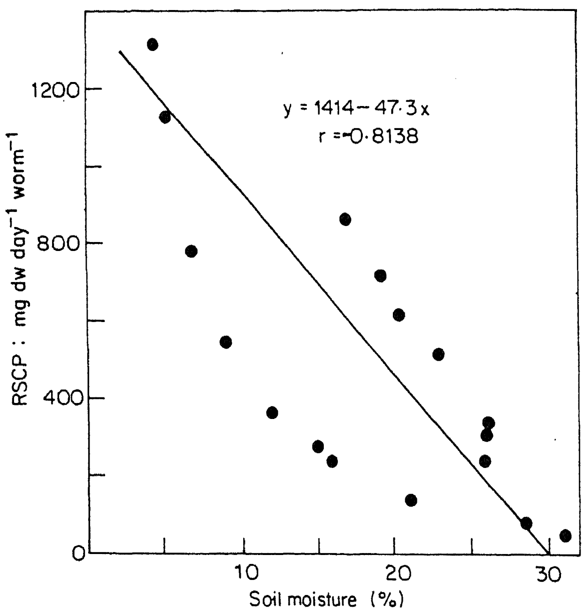

Figure 1. Relationship between the soil moisture content and RSCP by the earthworms of the selected sites. RSCP is the average of all species occurring in the site.

density and the moisture level at the field capacity of the site were not correlated (table 2). Similarly, no correlation between bulk density of the soil in a site and the worm density therein was found (table 2). However, the bulk density significantly increased the rate of cast production in a linear (figure 2, table 2) fashion. Clayey loams showed greater moisture content at field capacities. The moisture content at the field capacity of soils affected the rate of cast production non-linearly describing a perfect parabolic relationship (figure 3). In other words, the extremes of moisture levels at field capacity range tended to increase the surface rate of cast production (figure 3). Interestingly, a significant negative correlation was obtained between the

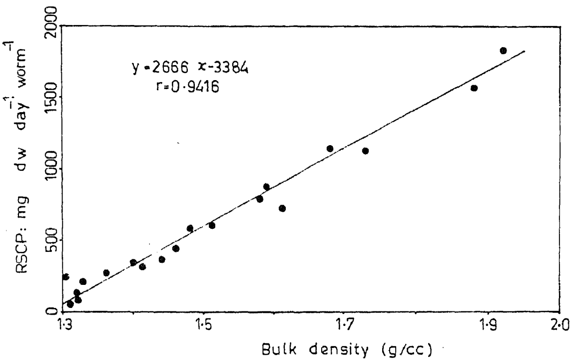

Figure 2. RSCP as a function of the bulk density of soils in the selected sites under field conditions. RSCP is the average of all species occurring in the site.

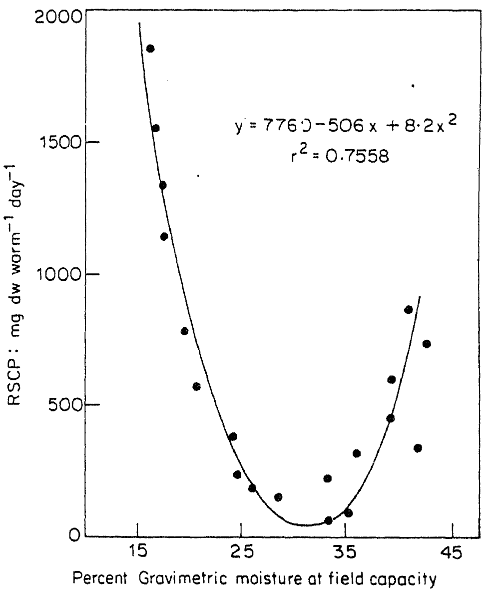

Figure 3. Relationship between the gravimetric moisture content at field capacity of soil in the site and the RSCP. RSCP is the average of all species occurring in the site.

worm density and the rate of cast production (table 2) indicating that higher population densities lowered the worm cast production.

The surface cast production in field conditions refers to a bulk of soil dwelling individuals, of various sizes, species and ecological categories. Therefore, the expression of rate of surface cast production on individual basis becomes an anachronic way of reporting the results. Moreover, a fairly large adult worm shows a greater biomass and casts more than a smaller worm with lesser biomass. Perhaps, for this reason, the rate of surface cast production showed a linear relationship with the biomass of the field population (tables 5, 6). The expression on biomass basis may not be a proper one to compare the rates of two different biotopes, as the biomass of worms is dependent on organic nutrient levels

Table 3. RSCP by various earthworm species of Karnataka, in a soil of bulk density, 1·462 g per cm³ under laboratory conditions (temp. 25°C).

	RSCP: mg dry wt. soil g^{-1} biomass*	
Species	Clitellates	Non-clitellates
Pheretima elongata	1672	798
Lampito mauritii	1381	536
Octochaetona pattoni	1293	NC
Pontoscolex corethrurus	998	464
Perionyx excavatus	825	692
Ocnerodrilus occidentalis	613	NC
Drawida barwelli	597	146
Octochaetoides beatrix	438	NC
Glyphidrilus annandalei	1786	526
Perionyx mysorensis	936	663

*Average of 8 experiments.
NC, Not left any surface casts.

Table 4. Threshold bulk density limits for surface casting by various earthworm species of Karnataka under laboratory conditions (temp. 25°C).

	Threshold bulk density (g per cm³)	
Species	Clitellates	Non-clitellates
Lampito mauritii	1·272	1·345
Pheretima elongata	1·216	1·462
Pontoscolex corethrurus	1·252	1·526
Ocnerodrilus occidentalis	1·423	NC
Octochaetoides beatrix	1·527	NC
Glyphidrilus annandalei	1·315	1·638
Perionyx excavatus	1·762	1·823
Perionyx mysorensis	1·826	1·842
Octochaetona pattoni	1·838	NC
Drawida barwelli	1·856	1·928

NC, Not left any surface casts.

(Krishnamoorthy 1985). However, in the present study a significant positive correlation was noticed between the density and biomass of mango-garden populations. The mango-garden populations have greater biomass than the wasteland populations (present results). The biomass of mango-garden populations increases with the worm density.

The confirmatory test conducted in the laboratory yielded fruitful results (tables 3, 4; figures 4, 5). The results of the test indicated that the rate of surface cast production is a function of the bulk density for a heterogenous batch of individuals, of various sizes, species and ecological categories. The heterogenous batch consisted of 8 species as listed in table 1 and included both clitellates and non-clitellates. Biomass, and crowding (density) of worms affected the rate of cast production (figure 4). Clitellates performed well in terms of surface cast production. The non-clitellates of 3 species viz. *Octochaetona pattoni*; *Ocnerodrilus occidentalis* Eisen and *Octochaetoides beatrix* Beddard never left any casts on the surface of soils with bulk density of 1·462 g/cm³ (table 3).

Table 5. Abundance and species distribution of earthworms in relation to soil type of wasteland biotopes in Karnataka [sites differed in agroclimatic conditions and surveys were made immediately after monsoon rain (Aug.–Sept. 1985). Sites and abundance have been arranged to emphasize the differences in species spectra].

Soil type of the site	No. of sites		Mean organic matter content (%)	Average soil (pH)	Total No. of species recorded	Average body weight g/ individual	Average density of worms (No./m^3)	Surface cast production (mg dry wt. day^{-1} $worm^{-1}$)	Percentage of sites with surface casts
	Examined	Noticed with surface casts							
Light sandy loam	39	27	11·1	6·8	8	1·4	168	1346	69
Black sandy loam	33	18	10·5	7·6	6	1·3	146	1152	55
Medium sandy loam	10	6	9·6	6·8	6	1·7	138	743	60
Brown sandy loam	60	38	9·8	7·2	5	1·2	114	971	63
Black loam	18	8	9·2	7·4	2	0·9	107	626	44
Gravelly loam	10	2	6·3	5·8	3	0·6	93	82	20
Brown clayey loam	20	8	7·9	6·4	4	0·7	72	310	40
Black clayey loam	15	6	8·2	7·6	3	0·5	66	254	40
Yellowish red gravelly loam	12	4	7·6	6·2	2	0·2	36	98	33
Clay	12	0	6·8	5·8	1	0·4	11	0	0
Heavy sand	18	0	1·7	7·2	0	0	0	0	0
Acid soils	18	0	1·8	4·8	0	0	0	0	0

Table 6. Statistical comparisons of the data presented in table 5.

Comparisons	Model observed	Regression equation	Correlation coefficient (r)	Level of significance
Soil organic matter vs PSCP	No relationship	$y=0{\cdot}739-0{\cdot}039x$	$-0{\cdot}2424$	NS
Number of species vs PSCP	Linear	$y=0{\cdot}1406+0{\cdot}0641x$	0·6051	$P<0{\cdot}05$
Density of worms vs PSCP	Linear	$y=0{\cdot}1227-0{\cdot}0028x$	0·6115	$P<0{\cdot}05$
Soil pH vs PSCP	Linear	$y=0{\cdot}2125x-1{\cdot}0398$	0·6276	$P<0{\cdot}01$
Soil organic matter vs worm density	Linear	$y=31{\cdot}3273x-179{\cdot}6405$	0·9362	$P<0{\cdot}01$
PSCP vs RSCP	No relationship	$y=503{\cdot}1455-45{\cdot}2609x$	$-0{\cdot}0251$	NS
Density of worms vs RSCP	"	$y=193{\cdot}746+2{\cdot}9133x$	0·2404	NS
Number of species vs RSCP	"	$y=164{\cdot}07+76{\cdot}6818x$	0·4156	NS
Soil organic matter vs RSCP	Linear	$y=150{\cdot}06x-845{\cdot}27$	0·6876	$P<0{\cdot}01$
Soil pH vs RSCP	Linear	$y=342{\cdot}46x-1844{\cdot}29$	0·7344	$P<0{\cdot}01$
Soil organic matter vs species number	Linear	$y=0{\cdot}6996x-1{\cdot}9429$	0·8456	$P<0{\cdot}01$

NS, Not significant.

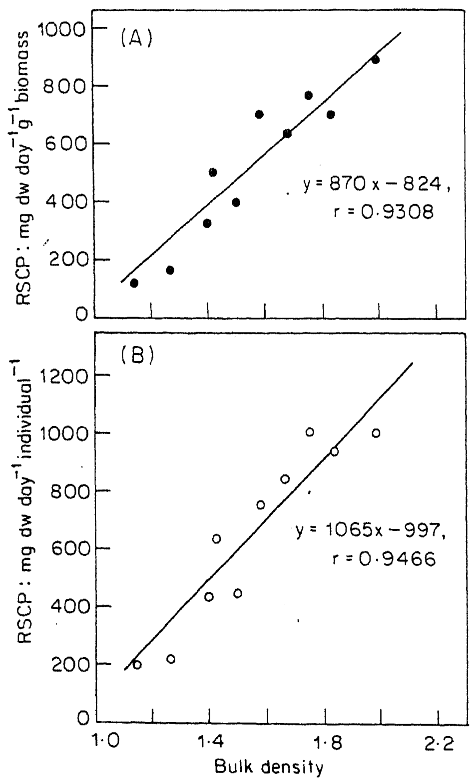

Figure 4. Effect of bulk density of experimental soils on the RSCP by (**A**) the biomass and (**B**) individual worm of a group of individuals of all sizes and species listed in table 1. RSCP is the average of 10 individual species listed in table 3.

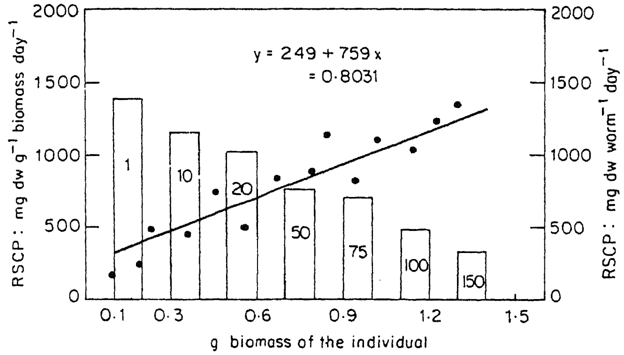

Figure 5. Effect of overcrowding of clitellate worms of *Lampito mauritii* (□) and biomass of the individual (●) on the RSCP in an experimental soil block with a bulk density of 1·368 g per cm^3. Numbers in histograms are the worms used in a volume of 1140 cm^3.

Compaction or threshold limits to induce casting at the surface were found to differ between species as well as between clitellates and non-clitellates (table 4). Non-clitellates of a majority of species behaved as surface casters excepting those of the 3 species mentioned earlier. Clitellates of *Pheretima elongata* E Perrier and *Glyphidrilus annandalei* Michaelsen casted at the surface even in soils with bulk densities as low as 1·116 g/cm^3 (table 4).

According to the present results the extrapolated figures of annual cast production at the surface in the biotopes (i.e. mango gardens) vary widely i.e. from 1·85–14·61 kg m^{-2} yr^{-1}. Obviously these gardens differ in many edaphic and agroclimatological characteristics, which would presumably account for variations in the production of surface casts.

Table 5 compares the data on the abundance of worms in different wasteland soil types and their surface casting activity. The sites with light sandy loams were not only rich in organic matter but also had greater species diversity. Sites with sands and acid soils (pH 4·8) were not inhabited by worm populations. Clayey sites showed only one species; that too in poor numbers. Sandy loams were colonized better, density- as well as diversity-wise than the clayey loams. Irrespective of soil type, a significant ($P < 0{\cdot}001$) positive correlation was obtained between the organic matter content and the worm density, and also between organic matter content and species diversity of the worms in the site. The surface casting occurred only in a few sites; the percentage never exceeded 63 (table 5).

In wasteland biotopes of Karnataka, the abundance and species distribution of earthworms vary with reference to soil type of the site (table 5). RSCP and percentage of surface cast production (PSCP) in the sites also vary with reference to soil type. Linear relationships found between (i) number of species and PSCP, (ii) worm density and PSCP, (iii) soil pH and PSCP, (iv) soil organic matter and worm density, (v) soil organic matter and RSCP, (vi) soil pH and RSCP and (vii) soil organic matter and species number in the site were statistically significant (table 6). The percentage of sites with surface cast production did not bear any relationship with the soil organic matter content of them. Similarly no relationships were found between PSCP and RSCP, worm densities and RSCP, and number of species and RSCP (table 6). These relationships further confirm that surface cast production is probably influenced by some soil physical factors. The variation in

percentage of sites with surface cast production could be due to variations existing in bulk density and field capacity of soils of the sites. In mango gardens there is a linear correlation between worm density and RSCP; but this is lacking in wasteland biotopes. The reason for this must be sought in the differences existing in the edaphic factors of wastelands.

In order to cross check whether the rate of surface casting differs with species, and to find out which of the species occurring in Karnataka casts more, an indirect laboratory assessment was attempted. Cast output of soil by the worm's gut is often helpful in assessing the rate of cast production. In other words, it is nothing but the faeces production.

Under laboratory conditions, in a bulk density of 1·6625 g/cm^3, when the cast outputs of soil by 8 species of worms were compared (figure 6) a positive correlation ($r = 0{\cdot}9408$) was obtained between the output and the relaxed body length of the species. If relaxation length is a species-specific character, the cast output can also be considered as a species-specific behaviour.

4. Discussion

Three noteworthy observations can be made out of the results viz. (i) the factors affecting the surface cast production, (ii) the casting behaviour of the worms and (iii) variations in the densities and body size of worms inhabiting wasteland biotopes. Among abiotic factors, the moisture content, bulk density and field capacity of soils were found to have immense influence over the surface cast production. The biotic factors like the crowding of the worm and maturity of worms influenced the surface cast production significantly. Tropical worms show high casting rates and this activity may result in the burial of litter and in a change in the environment in which decomposition takes place, probably by enhancing the microbial activity and nutrient fluxes (Cook *et al* 1980).

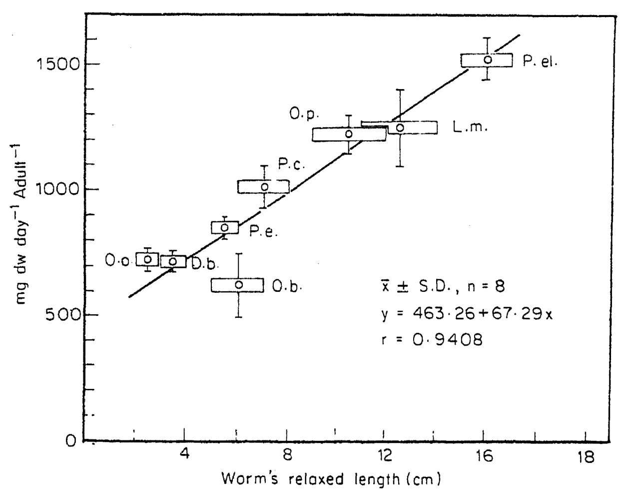

Figure 6. Cast output (mean ± SD) of experimental soil through a worm's gut as a function of the relaxed body length of the worm. Horizontal bar indicates the variations in the relaxed body length among the individual worms studied. Abbreviations as in table 1:

All the species of the present study are night-casters. Faeces production is nearly 2–50 times higher in tropics than in temperates (Lee 1983), but a low proportion of the ingested soil is rejected as surface casts. However, the surface casting rate in tropics is not as high as in temperates (Kollmannsperger 1956). A major part of the faecal casts is left in the subsoil burrows itself. In fact the true amounts involved must be much greater than the surface casts in cultivated soils where large quantities of casts will be required to line the burrows and to fill the soil interstices. Lavelle (1984) estimated that the surface casts form only 25% of the total casts produced; the rest is hidden in the sub-soil layers and burrows. Soil temperature, organic matter, humidity and environmental temperature are known to influence the total casting activity (Evans and Guild 1947; Jefferson 1958; Edwards and Lofty 1977). Worms prefer to occupy such environments where these requirements are optimal (Satchell 1967; Kale and Krishnamoorthy 1981; Senapati *et al* 1980). Such a choice will have an impact on the active cast production, soil nutrient turnover, soil humification and turnover of exchangeable ions, bases and nitrogen (Edwards and Lofty 1977; Hartenstein 1983).

Worm density and soil consuming capacity of the worms contribute greatly to the cast production. In tropics the casting activity is limited to certain seasons (Edwards and Lofty 1977; Dash and Patra 1977; Dash 1978; Krishnamoorthy 1985). Whether the soil type affects the cast production is not known. But it is known to affect the qualitative and quantitative distribution of populations (Guild 1955; Satchell 1967; Edwards and Lofty 1977). Light and medium loams have greater abundance than heavier clays or open gravelly sands (Guild 1955). The present results corroborate this observation.

Soil compaction over threshold limits, which may be a result of trampling by human and vehicles. (Piearce 1984) and raising sheep density in pastures (Hutchinson and King 1980), has a declining effect on the abundance and biomass of earthworms (Bostrom 1986). Threshold limits to induce surface casts vary with species and maturity of worms (present results). Worms which are supposedly non-surface casters are reported to produce surface casts on compact silts both in the laboratory (Thompson and Davies 1974; Habibullah and Ismail 1985) as well as in the field (Piearce 1984). Compact soils have high bulk density (Brady 1974). The number of casts increases with soil bulk density, organic matter and fine silt particles (Thompson and Davies 1974). The same is noticed in the present study.

Rainfall (Krishnamoorthy 1989) and soil moisture (Reynoldson 1966) are known to affect the abundance of earthworms. Soil moisture affects the distribution and abundance (Edwards and Lofty 1977), biomass (Lakhani and Satchell 1970), and survival and growth of populations (Dash and Patra 1977; Dash 1978). It is clear from the present results that soil moisture and moisture level at field capacity have direct effects on surface casting. Increase in rainfall or moisture influences the worm populations in various ways: (i) by increased litter supply, (ii) by enhancing the quality of litter due to increased microbial activity, (iii) by facilitating the worm movements and thereby accelerating growth and reproduction and (iv) by reducing the mortalities of juveniles and adults which occurs normally as a direct result of desiccation. The present results show that soil moisture besides the bulk density and field capacity affect the casting activity.

The cast output or faeces production seems to be dependent on the consummatory behaviour of worm, soil nutrient, nature of soil organic matter, and

the physical characteristics of soil (Satchell 1967; Hartenstein 1983). The cast output in *Lampito mauritii* Kinberg has been correlated with the volume of soil ingested (Habibullah and Ismail 1985). The present results are particularly interesting as they reveal that the cast output of soils increases with the relaxed length of the worm species, and the latter differ greatly among tropical species. The tropical worms especially of the present study, have higher cast outputs ($P<0{\cdot}001$) than the temperate species (cf. present results with those of Satchell 1967; Crossley *et al* 1971). It is probable that the cast output is a species specific function rather than a consummatory response.

Wastelands of Karnataka are also inhabited by worms depending on the type, pH and organic matter content of the soils existing there. Surface cast production was also noticed in such biotopes. Wasteland worms differ with those of agro-biotopes (like mango gardens) in having a poor biomass.

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